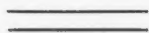


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## CONTENTS

Inheritance of post-harvest seed dormancy and kernel colour in spring wheat lines	F. Gfeller and S. G. Gfeller	1
Sawfly resistance in wheat. III. Changes in resistance during the development of the wheat plant	- D. W. A. Roberts	7
Management of medium red clover for seed and hay production	J. E. Winch and W. E. Tossell	21
The influence of the host on oviposition by the wheat stem sawfly, <i>Cephus cinctus</i> Nort. (Hymenoptera: Cephidae)	- N. D. Holmes and L. K. Peterson	29
Effects of gibberellin treatments on germination of various species of weed seeds	Wm. G. Corns	47
A study of the relationship between the amount of bloom and yield of apples	R. P. Longley	52
Factors influencing the colour of potato chips	- L. R. Townsend and G. W. Hope	58
The influence of variety, temperature, and stage of growth on the response of spring barley to photoperiod	- A. A. Guildard	65
The use of cereal grains as companion crops in dryland forage crop establishment	M. R. Kilcher and D. H. Heinrichs	81
Cold hardness of sprouting wheat as affected by duration of hardening and hardening temperature	- J. E. Andrews	94
Two virus diseases of rhubarb in eastern Ontario	- D. S. MacLachlan	104
La transmission par les insectes de <i>Corynebacterium sepedonicum</i> (Spieck. & Kott.) Skaptason et Burkholder	Joseph Duncan et Henri G��n��reux	110
Mercury residues on apple fruit and foliage	- R. G. Ross and D. K. R. Stewart	117
The dry matter digestion <i>in vitro</i> of forage crops	- K. W. Clark and G. O. Mott	123
Variety, fertilizer, management interactions in alfalfa	- B. E. Twamley	130
Infection of wheat with <i>Helminthosporium sativum</i> in relation to the nitrogen content of the plant tissues	- P. M. Simmonds	139
Fertilizers and the nutritive value of wheat grown on a sulphur-deficient grey wooded soil	C. F. Bentley, J. A. Carson and J. P. Bowland	146
Control of the onion maggot, <i>Hylemya antiqua</i> (Meig.) (Diptera: Anthomyiidae), with insecticides in organic soils of southwestern Quebec	- J. P. Perron and J. Lafrance	156
Chemical control of the raspberry root borer, <i>Bembecia marginata</i> (Harr.), on loganberry in British Columbia	- J. Raine and H. Anderson	160
Soil insecticides for control of the tuber flea beetle, <i>Epitrix tuberis</i> (Gent.) in the interior of British Columbia	- F. L. Banham	165
Sprinkler irrigation as a means of applying soil insecticides for controlling the tuber flea beetle, <i>Epitrix tuberis</i> (Gent.), in the interior of British Columbia	- F. L. Banham	172
Insecticide row treatments for the control of wireworms in potatoes	- R. H. Burrage	178
Influence of periodic shading on the length and solidness of the internodes of Rescue wheat	N. D. Holmes, R. I. Larson, I. K. Peterson and M. D. MacDonald	183
The influence of the extract of some crops and soil residues on germination and growth	K. F. Nielsen, T. F. Cuddy and W. B. Woods	188
Effects of Sevin on phytophagous mites and predators in an Ontario peach orchard	Wm. L. Putman and D. C. Herne	198
<i>Notes on:</i>		
The occurrence of binucleate pycniospores in species of <i>Puccinia</i>	- R. C. McGinnis	202
"Frontier" reed canary grass ( <i>Phalaris arundinacea</i> L.)	- R. M. MacVicar	203
Climatic trends in the lower Peace River region of northern Alberta	T. C. Edmonds and C. H. Anderson	204
Merit soybean	- F. Dimmock	207
A plot irrigator	Chas. Walkof	209

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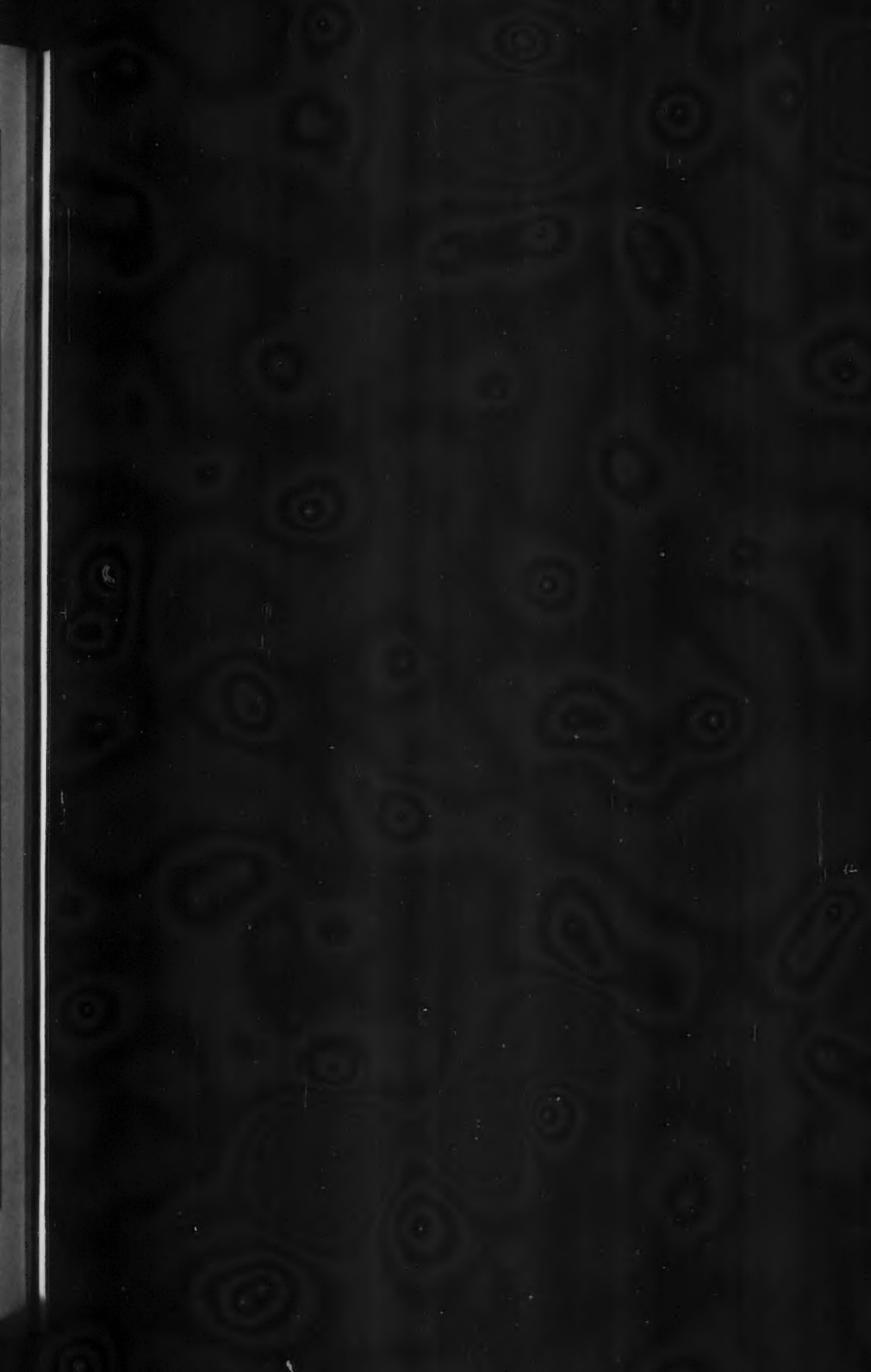
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# CANADIAN JOURNAL OF PLANT SCIENCE

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## INHERITANCE OF POST-HARVEST SEED DORMANCY AND KERNEL COLOUR IN SPRING WHEAT LINES<sup>1</sup>

F. GFELLER AND F. SVEJDA

*Canada Department of Agriculture, Ottawa, Ontario*

[Received for publication February 9, 1959]

### ABSTRACT

The inheritance of post-harvest seed dormancy was studied in F<sub>7</sub> lines of a cross between Renown—a dormant red spring wheat, and Cascade—a non-dormant white spring wheat. The estimate of heritability of seed dormancy was 73 per cent. The inheritance of seed coat colour was controlled by three pairs of duplicate genes. Red seed coat colour was found to be associated with seed dormancy. All lines with white seed coat colour were non-dormant. The moisture content of the seed was found to influence seed dormancy. Although moisture affected the dormancy status of the seed, other factors appeared to be involved as significant differences between lines of red seed coat colour were obtained at similar moisture levels. It is postulated that the degree of seed dormancy is controlled by the multiple genes which govern seed colour. The inheritance of phenol colour reaction of the seed was monogenic, and no association was found between seed dormancy and phenol colour reaction.

### INTRODUCTION

The term seed dormancy denotes any condition inherent in the seeds which prevents the germination of viable seeds for a definite period after harvest. A period of seed dormancy in wheat is advantageous because sprouted seed is undesirable for milling, and its value as seed is reduced owing to a loss of viability during storage (7, 8, 9, 15). Harrington (9) made a survey of Canadian cereal varieties with respect to seed dormancy and found that varieties within crops differed with respect to their dormancy.

The present study of seed dormancy was prompted by the frequent occurrence of sprouting in the stock of the variety Cascade grown in Eastern Canada. This variety was licensed in 1947, and distributed in 1948 as an improved, high-yielding white spring wheat, and was the first white spring wheat grown to any extent in Eastern Canada. During wet seasons, seed of Cascade sprouted in the field, and during threshing and storage a further deterioration of the seed occurred, thus reducing germination.

Several authors have shown that dormancy is a heritable character readily recovered in hybrid populations (2, 5, 6, 7). Other investigators (2, 10, 13, 18) associated dormancy with seed colour and showed that white

<sup>1</sup>Contribution No. 241 from the Cereal Crops Division, Canada Department of Agriculture, Ottawa, Ont.

wheats were the least resistant to sprouting. Under field conditions, during wet season, the sprouting of seed often occurs in the spike of non-dormant wheats and there is a correlation between the dormancy in threshed seeds and that occurring of seeds in unthreshed spikes (9). It was shown (18) that physical characteristics such as the manner in which the glumes cover the seed and the morphological structure of the glumes themselves may retard sprouting in the spikes, particularly if the water supply is not optimal. Wellington (18) showed that a lower moisture in the seed tended to be related to lower dormancy. Larson *et al.* (11) reported that seed storage temperatures of 30 to 35° C. were more conducive to breaking seed dormancy than temperatures below this level.

The present paper deals with the inheritance of seed dormancy and its relationship to seed coat colour, seed moisture, and phenol colour reaction in the seed in a cross between Cascade—a non-dormant white sprig wheat, and Renown (7)—a dormant red spring wheat.

#### MATERIALS AND METHODS

The cross between Cascade and Renown was made in the greenhouse during 1949-50. Four hundred  $F_2$  plants were grown and three seeds were taken from each  $F_2$  plant. Only one plant was harvested at random out of each line and again three seeds were planted for each line in the following generation. This procedure was followed until the  $F_6$  generation when seed from three plants in each line was bulked to produce the  $F_7$  lines. A random sample of 188  $F_7$  lines and three replications of each parent were grown in 6-foot rows in the field during 1958. Both parents were grown as controls at a 30-row interval. The centre 4-foot section of each row or line was harvested at the ripe stage. Each line was tied into a sheaf and these were dried for 3 days in the greenhouse before threshing. As soon as the sheaves were threshed moisture determinations were made on 2-gram samples of seed and the remainder stored in plastic bags to prevent excessive drying while duplicate samples were prepared for germination. The per cent moisture of 188 lines ranged from 10 to 23 per cent in the first test.

Another germination test was made 10 days later, after additional drying of the seed. Only 88 lines out of the 188 lines were used in this test and the moisture was reduced to 11 to 14 per cent. These 88 lines were used later in the analysis of germination percentages.

The procedure followed for the moisture determinations was the "Air-Oven Method" (3). All germination tests were carried out at the Plant Products Division, Ottawa, in temperature-controlled cabinets. A relative humidity of 80 to 100 per cent and a temperature of  $20^\circ \pm 1^\circ$  C. were maintained during the germination period of 7 days. Duplicate samples of 100 seeds each were counted with the vacuum head counter (12), and placed between water-saturated blotting paper. At the end of the 7-day germination period all seeds failing to sprout were removed and placed in a chamber maintained at  $5^\circ \pm 1^\circ$  C. Any seeds not sprouted after 3 days in this second phase were regarded as non-viable. The difference in germination at  $20^\circ$  C. and at  $5^\circ$  C. was considered to be due to

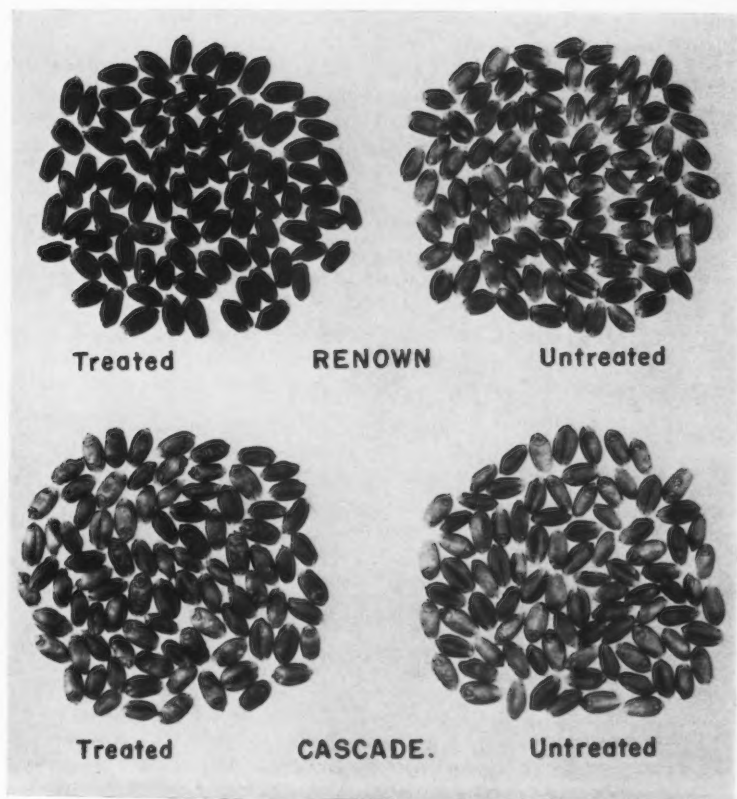
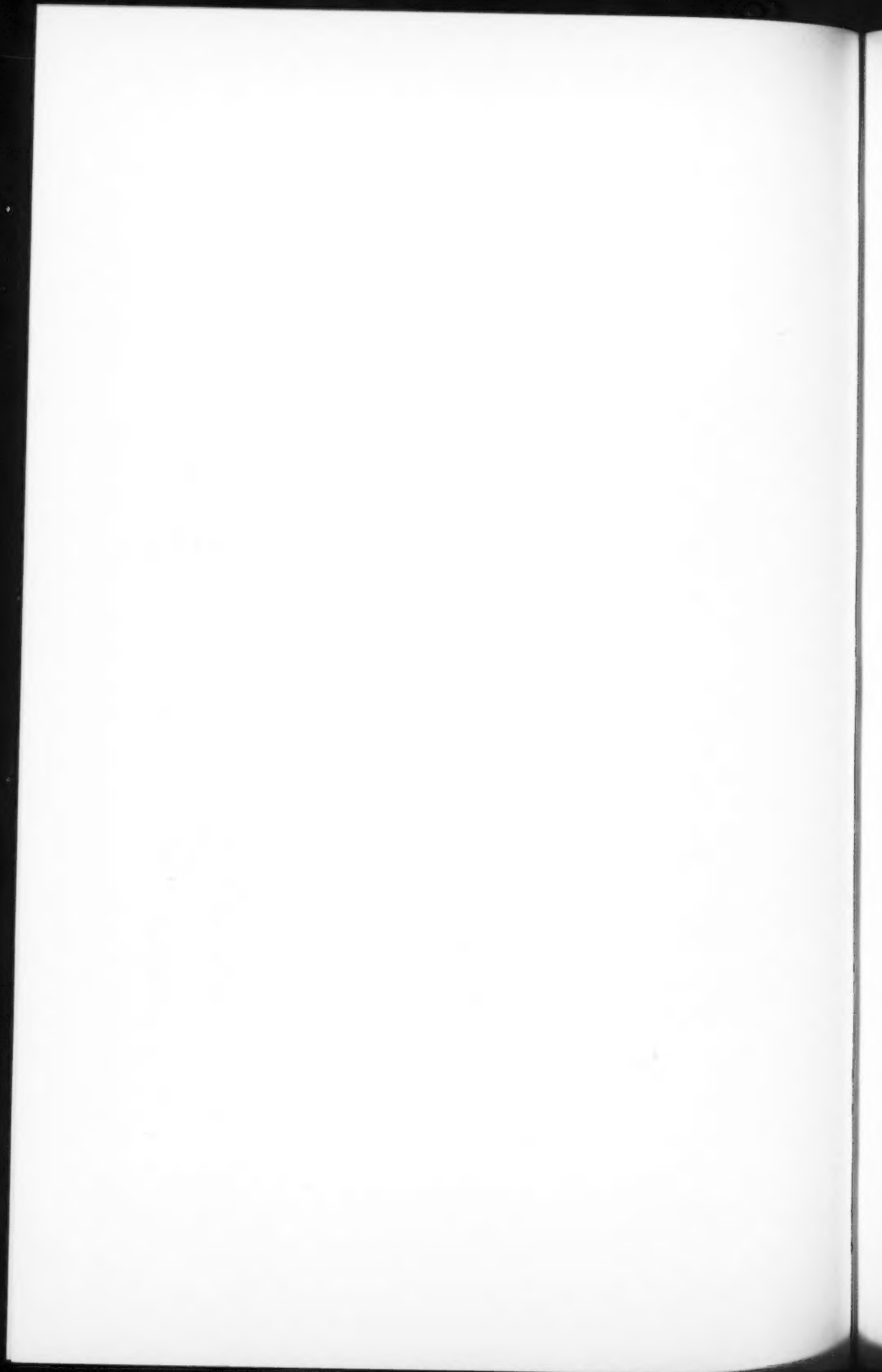


FIGURE 1.







seed dormancy. The percentage germination was based on the total number of viable seeds. Seeds were classified as having germinated when the green leaves extended over half-way up the sheath of the coleoptile (1).

The seed colour and the phenol colour reaction was determined on  $F_7$  lines. The phenol colour reaction of the kernels was obtained by the procedure recorded by Fraser and Gfeller (4).

### RESULTS AND DISCUSSION

The genetic analysis in this study, involving several characters, i.e. phenol kernel colour, seed coat colour and seed dormancy, uses homozygous line ratios rather than the usual  $F_2$  plant and  $F_3$  line ratios. Theoretically, a random propagation of each  $F_2$  plant when carried to the  $F_7$  should give two different homozygous genotypes for one independent factor pair and four different genotypes for two independent factor pairs etc. This method was proposed by C. H. Goulden\* for use in inheritance studies of quantitative characters in spring wheat. In this method the importance of complete randomization is evident.

To prove the accuracy of the data on post-harvest seed dormancy it was necessary to show that a random propagation of each  $F_2$  plant occurred.

From the data recorded in Tables 1 and 2 it appears that the propagated  $F_2$  lines represent a random sample and, therefore, establish confidence in the use of the data for post-harvest seed dormancy studies.

\*Personal communication.

TABLE 1.—AGREEMENT OF FIT TO A 1:1 RATIO FOR PHENOL COLOUR

Genotypes	Observed	Calculated	O-C	$\frac{(O-C)^2}{C}$
Black-brown (PK PK)	55	56.5	-1.5	.0039
Light-brown (pK pK)	58	56.5	+1.5	.0039
Total	113	113.0	0.0	$X^2$ .0078 P = .90

A value of  $X^2$  of .0078 and with P .90 indicates a good agreement with the single gene hypothesis.

TABLE 2.—AGREEMENT OF FIT TO A 7:1 RATIO FOR SEED COAT COLOUR

Genotypes	Observed	Calculated	O-C	$\frac{(O-C)^2}{C}$
Red	162	164.5	-2.5	.038
White	26	23.5	+2.5	.266
Total	188	188.0	0.0	$X^2$ .304 P = .6

The good fit obtained for such simply inherited characters as phenol colour reaction and seed coat colour are reported in Tables 1 and 2.

The phenol colour reaction of the red seeded Renown wheat was black-brown, and that of Cascade, a white wheat, was light-brown (Figure 1).

On the basis of one pair of independently inherited genes, assuming two colour classes only, a 1:1 ratio in  $F_7$  lines is expected. The genetic data obtained are recorded in Table 1.

For seed coat colour a ratio of 7 red lines to 1 white was obtained for the homozygous genotypes. A good agreement of fit for this 3-factor ratio is indicated by the  $X^2$  value of .304 and the P value of .6. Since intensity of seed coat colour is associated with the vitreous aleurone layer of the endosperm (14), it was not possible to distinguish between lines which carried one or more dominant genes for seed coat colour.

The percentage moisture of the seed samples varied considerably between hybrid lines. A correlation coefficient of  $r = .2953$  was obtained for percentage moisture and germination percentage of red-seeded lines. This value exceeds the 1 per cent level of probability indicating that moisture levels influence dormancy trials.

The data for percentage germination in both red and white hybrid lines are given in Table 3.

TABLE 3.—AVERAGE PER CENT GERMINATION OF WHITE- AND RED-SEEDED LINES OBTAINED AT 20° C.

Colour	Number of lines	Average germination in per cent
Red	162	51
White	26	81

The  $X^2$  for independence between seed colour and germination was 85.11 and P .001, indicating an association between seed coat colour and germination percentage.

The germination data were subjected to an analysis of variance. In this analysis (Table 4) all significant F values exceeded the 1 per cent probability level. The highly significant F value obtained between the germination tests indicates that a 10-day storage period tended to increase the per cent germination. Therefore, to establish which factors contributed to seed dormancy the following subdivisions were made for the detailed statistical analysis:

1. Hybrids with red seeds, and less than 14 per cent moisture.
2. Hybrids with red seeds, and more than 14 per cent moisture.
3. Hybrids with white seeds (no moisture limit).
4. Parents (no moisture limit).

Highly significant differences were obtained between red-seeded hybrids of different moisture levels, between red- and white-seeded hybrids and between hybrids and parents. Significant differences were also obtained within each group of red-seeded hybrids and between Renown.

TABLE 4.—ANALYSIS OF VARIANCE OF GERMINATION PERCENTAGES

Source of variation	DF	F
Tests	1	138.83**
Hybrids and parents	93	9.97**
Red hybrids with less than 14% moisture vs. red hybrids with more than 14% moisture	1	116.97**
Red vs. white hybrids	1	290.02**
Cascade vs. Renown	1	26.71**
Parents vs. hybrids	1	30.49**
Between hybrids of less than 14% moisture	36	8.60**
Between hybrids of more than 14% moisture	36	3.27**
Between white hybrids	13	1.52
Between Cascade	2	0.78
Between Renown	2	6.98**
Residuals	93	
Total	187	

\*\*Exceeds 0.01 probability level

TABLE 5.—ANALYSIS OF VARIANCE OF PER CENT GERMINATION AMONG PARENTS AND F<sub>7</sub> HYBRID LINES

Source of variation	DF	SS	MS
Tests	1	11936.19	
Between parents	1	2296.34	
Parents vs. hybrids	1	2621.70	
Parents vs. hybrids vs. tests	1	6.27	
Hybrids	87	73433.44	844.06 M <sub>1</sub>
Hybrids vs. tests	87	6796.88	78.13 M <sub>2</sub>
Parents vs. tests = 1\	9	2527.99	280.88 M <sub>3</sub>
Within plots of parents = 8\			
Total	187	99618.81	

$$\text{Heritability in per cent} = \frac{M_1 - M_2}{M_1 - M_2 + M_3} \times 100$$

The former were affected by differences in the genotype and moisture percentage whereas the latter varied because of difference in moisture level. Differences within groups of white-seeded hybrids and Cascade were not significant as moisture level exerted no influence on their germination.

The data for the pertinent components of variance known to influence heritability are given in Table 5.

A heritability of 73 per cent was obtained for seed dormancy. Although differences in intensity of red seed coat colour cannot be distinguished, the close association between red seed coat colour and dormancy as indicated by the high heritability provides an advantage in early generation selection for post-harvest seed dormancy.

## CONCLUSIONS

The data obtained in this study indicate that there is a complete association between red seed coat colour and dormancy. None of the white-seeded lines was dormant. A significant difference was obtained for degree of dormancy within red-seeded lines of similar moisture content. It is possible, therefore, that lines with a greater number of genes controlling seed coat colour may be the most dormant. The genes governing seed coat colour may also control dormancy as the data indicate a complete association between red seed coat colour and dormancy. The association or pleiotropic effects of genes governing seed coat colour and dormancy will be studied further.

## ACKNOWLEDGEMENT

The authors wish to express their appreciation to P. F. Cuddy of the Plant Products Division, Ottawa, for the interest taken in this dormancy study and for the use of the germination cabinets, and to M. Waldron, Student Assistant, for the work and preparation required in the assessment of seed dormancy. Acknowledgement is also made to H. Miller, of the Cereal Crops Division, who was responsible for the moisture determinations.

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## SAWFLY RESISTANCE IN WHEAT

### III. CHANGES IN RESISTANCE DURING THE DEVELOPMENT OF THE WHEAT PLANT<sup>1</sup>

D. W. A. ROBERTS

Canada Department of Agriculture, Lethbridge, Alberta

[Received for publication February 12, 1959]

#### ABSTRACT

Quantitative data obtained in field experiments showed that the resistance of wheat to attack by the wheat stem sawfly (*Cephus cinctus* Nort.) depended on the stage of development of the plants at the time of oviposition. Wheat plants were usually most heavily infested for a part or all of the period from 1 week before shot blade to 1 week after the flowering stage. Rescue, H46146, H4191, Golden Ball, and Melanopus lost their resistance to the development of the eggs and first-instar larvae some time between shot blade and flowering. Thatcher and Red Bobs did not show this type of resistance. The mortality of the older larvae increased in plants infested toward maturity. The time at which this increase began depended on the variety involved and ranged from just before shot blade to just after flowering.

#### INTRODUCTION

In an earlier paper (5) in this series it was shown that there were at least three types of resistance of wheat to the wheat stem sawfly, *Cephus cinctus* Nort. These were: (a) resistance to egg laying; (b) resistance to development of eggs and establishment of first-instar larvae, and (c) resistance to the development of the older larvae. The three types were based on differences between varieties in (a) percentage of stems not infested, (b) percentage of infested stems with oviposition scars, and (c) percentage of tunnelled stems not cut. In the present paper the changes in these types of resistance that accompany the development of the wheat plant are described.

Three types of experiments were conducted to study the changes in resistance. In the first experiment plants were seeded on a single date and exposed to infestation by sawflies at weekly intervals. The changes in resistance observed in this type of experiment may have been caused by either changes in the stage of development of the plants at the time of infestation or seasonal changes in the environment that may have affected the behaviour of the insect or the response of the plant. To distinguish between the two possibilities the second type of experiment was used. This consisted in exposing the plants from several different dates of seeding to infestation by sawflies on one or more dates. Unfortunately, this type of experiment was hampered at Lethbridge by the short growing season and the tendency of the plants sown later in the season to catch up with the earlier sown plants in development. Consequently the experiments did not give a conclusive answer. This was the reason for doing the third type of experiment in the greenhouse in the winter-time. In this test one variety of wheat was seeded on a single date each year and exposed to infestation by sawflies at weekly intervals.

<sup>1</sup> Contribution from the Plant Pathology Section, Research Station, Lethbridge, Alta.

### MATERIALS AND METHODS

During the years 1950-53, plot experiments were carried out in areas that were almost free from sawflies.

Thatcher, Red Bobs, Rescue, H46146, H4191, Golden Ball, and Melanopus were chosen for the tests for the reasons explained previously (5).

The cages used in these experiments were 36 inches high and covered an area 24 by 26 inches. They were screened with 20-mesh, natural colour, Lumex. The light intensity inside the cages was approximately 70 per cent of the intensity outside.

Wheat stubs containing sawfly larvae were collected from the Lethbridge district in the spring and stored at 10° C. Three weeks before sawflies were needed the stubs were moved to 25° C., where they remained until the adults emerged. An aspirator was used to transfer male and female sawflies into separate groups of 25 of each sex in flasks containing excelsior. In some cases these sawflies were stored for a few days in this condition at 10° C. before use.

#### *Experiments with One Date of Seeding*

Wheat was sown in rows spaced 6 inches apart on a single date each year (Table 1) and exposed to infestation at weekly intervals after stem elongation had begun. Each week two plot areas of each variety were chosen systematically to conserve space and facilitate anchoring the cages. The areas were caged separately, and 25 male and 25 female sawflies were released inside each of the two cages. The areas between the cages served as controls to check for the presence of a wild population.

TABLE 1.—DATES ON WHICH WHEAT WAS SEEDED AND FIRST EXPOSED TO INFESTATION BY WHEAT STEM SAWFLY

Variety	Date of seeding	Date of first exposure to infestation
Thatcher	May 8, 1950	June 17
Rescue		
Thatcher	May 8, 1951	June 19
Rescue		
Thatcher	May 6, 1952	June 19
Red Bobs		
Rescue	May 13, 1953	June 26
Thatcher		
Red Bobs	May 13, 1953	June 26 <sup>1</sup>
Rescue		
H46146	May 13, 1953	June 23
H4191		
Golden Ball	May 13, 1953	June 23
Melanopus		
Thatcher	May 13, 1953	June 23
Red Bobs		
Rescue	May 27, 1953	July 6, 8 <sup>2</sup>
Thatcher		
Red Bobs	June 16, 1953	July 6, 8 <sup>2</sup>
Rescue		

<sup>1</sup> Red Bobs infested June 23, 30, July 7, 17, 24, 31, August 7, and 13.

<sup>2</sup> These dates count as second week.

TABLE 2.—SUMMARY OF ANALYSIS OF VARIANCE OF DATA ON THE EFFECT OF TIME OF EXPOSURE TO INFESTATION BY WHEAT STEM SAWFLY ON THE RESISTANCE OF THREE VARIETIES OF WHEAT SOWN ON A SINGLE DATE OF SEEDING EACH YEAR

Variety	Years tested	Averages of transformed <sup>1</sup> percentage values for sawfly resistance										L.S.D. 5%	Level of significance (%)		
		Time of exposure to infestation, weeks from shot blade											Weeks	Years	Weeks x years
		-2	-1	0	+1	+2	+3	+4	+5						
Stems not infested															
Thatcher	4	70	58	49	48	42	54	72	74		11	1	—	—	1
Red Bobs	2	—	44	39	42	57	66	78	77		12	1	—	—	—
Rescue	4	74	58	50	55	48	60	78	—		14	5	—	—	1
Infested stems with oviposition scars															
Thatcher	4	18	13	8	10	7	14	14	22		—	—	1	—	—
Red Bobs	2	—	6	6	3	27	9	26	26		—	—	—	—	—
Rescue	4	54	52	51	29	16	16	25	—		13	1	1	—	—
Tunnelled stems not cut															
Thatcher	4	35	34	22	45	52	70	74	69		13	1	1	—	5
Red Bobs	2	—	29	27	35	60	71	61	73		21	1	—	—	—
Rescue	4	47	35	36	58	75	86	70	—		21	1	—	—	5

<sup>1</sup> Inverse sine transformation



In 1950 and 1951 the cages were left in place for only 1 week, but in 1952 and 1953 they were left in place for 2 weeks. This change was made because the control samples from the 1951 test on Red Bobs were infested and it was thought that the source of infestation was sawflies escaping when cages were moved at the end of 1 week.

### *Experiments with Different Dates of Seeding*

In these experiments the wheat was sown on dates separated by at least 2-week intervals. Seeding and exposure to infestation were carried out as described above on the dates indicated in Tables 1, 4, and 5. Systematic exposure of the plots to sawflies in 1952 was prevented by a shortage of sawflies, caused by an unexpectedly small proportion of females emerging from the stubs.

Whenever sawflies were liberated inside the cages, specimen wheat plants were taken and pressed for reference purposes. Thus it was possible to compare the stage of development of a given variety from test to test and of the different varieties on any given date of exposure to infestation.

TABLE 3.—EFFECT OF TIME OF EXPOSURE TO INFESTATION BY WHEAT STEM SAWFLY ON THE RESISTANCE OF SEVEN VARIETIES OF WHEAT SOWN ON A SINGLE DATE OF SEEDING IN 1953

Variety	Time of exposure to sawfly attack, weeks after first exposure to attack								
	0	1	2	3	4	5	6	7	8
Percentage of stems not infested									
Thatcher	92	63	57 <sup>1</sup>	66	51 <sup>2</sup>	50	97	95	—
Red Bobs	87	66	29 <sup>1</sup>	43	89 <sup>2</sup>	72	96	98	—
Rescue	86	43	37 <sup>1</sup>	48 <sup>1</sup>	54 <sup>2</sup>	42	97	98	—
H46146	93	73	60	84 <sup>1</sup>	46 <sup>2</sup>	32 <sup>2</sup>	87 <sup>2</sup>	88	98
H4191	99	69	48	79 <sup>1</sup>	76	29	42 <sup>2</sup>	86 <sup>2</sup>	99
Golden Ball	97	86	69	90 <sup>1</sup>	49	12 <sup>2</sup>	40 <sup>2</sup>	83	—
Melanopus	95	76	58	85 <sup>1</sup>	19	20 <sup>2</sup>	47 <sup>2</sup>	51	97
Percentage of infested stems with oviposition scars									
Thatcher	10	1	1 <sup>1</sup>	1	1 <sup>2</sup>	1	7	10	—
Red Bobs	5	4	0 <sup>1</sup>	1	3 <sup>2</sup>	1	23	17	—
Rescue	22	23	21 <sup>1</sup>	19 <sup>1</sup>	3 <sup>2</sup>	2	11	—	—
H46146	58	35	69	36 <sup>1</sup>	32 <sup>2</sup>	10 <sup>2</sup>	5 <sup>2</sup>	9	—
H4191	—	38	17	49 <sup>1</sup>	4	5	1 <sup>2</sup>	3 <sup>2</sup>	—
Golden Ball	—	17	19	38 <sup>1</sup>	13	5 <sup>2</sup>	4 <sup>2</sup>	5	—
Melanopus	31	20	16	8 <sup>1</sup>	2	0 <sup>2</sup>	0 <sup>2</sup>	2	—
Percentage of tunnelled stems not cut									
Thatcher	43	33	16 <sup>1</sup>	15	58 <sup>2</sup>	96	93	100	—
Red Bobs	49	29	23 <sup>1</sup>	39	74 <sup>2</sup>	93	90	100	—
Rescue	25	18	20 <sup>1</sup>	48 <sup>1</sup>	91 <sup>2</sup>	100	100	—	—
H46146	63	51	37	41 <sup>1</sup>	86 <sup>2</sup>	97 <sup>2</sup>	97 <sup>2</sup>	97	—
H4191	—	31	24	60 <sup>1</sup>	86	82	97 <sup>2</sup>	100 <sup>2</sup>	—
Golden Ball	—	60	46	60 <sup>1</sup>	58	67 <sup>2</sup>	95 <sup>2</sup>	100	—
Melanopus	11	39	41	38 <sup>1</sup>	56	68 <sup>2</sup>	88 <sup>2</sup>	99	—

<sup>1</sup> Plants in shot blade at this time

<sup>2</sup> Plants flowering at this time



TABLE 4.—EFFECT OF DATES OF SEEDING AND STAGE OF DEVELOPMENT OF WHEAT PLANTS AT TIME OF EXPOSURE TO INFESTATION ON SAWFLY RESISTANCE IN 1952

Date seeded	Date infested	Stage of development at time of exposure to infestation	Thatcher			Red Bobs			Rescue		
			Stems not infested	Infested stems with oviposition scars	Tunnelled stems not cut	Stems not infested	Infested stems with oviposition scars	Tunnelled stems not cut	Stems not infested	Infested stems with oviposition scars	Tunnelled stems not cut
			%	%	%	%	%	%	%	%	%
June 27	Aug. 88	Shot blade	59	14	29	67	14	42	88	29	40
June 27	Aug. 12	Shot blade	59	6	37	52	1	53	70	55	79
May 6	July 4	Boot	49	2	47	65	0	50	71	33	90
June 14	Aug. 8	Past flowering	77	22	84	59	0	82	24	13	87
June 14	Aug. 12	Past flowering	33	4	71	62	0	87	65	6	73
May 22	Aug. 8	Beginning to ripen	99	—	—	100	—	—	99	—	—
May 6	Aug. 8	Almost ripe	100	—	—	98	—	—	99	—	—

TABLE 5.—EFFECT OF DATES OF SEEDING AND STAGE OF DEVELOPMENT OF WHEAT PLANTS AT TIME OF EXPOSURE TO INFESTATION ON SAWFLY RESISTANCE IN 1953

Variety	Thatcher			Red Bobs			Rescue		
	May 13	May 27	June 16	May 13	May 27	June 16	May 13	May 27	June 16
Date of seeding									
Weeks after first exposure to infestation until—									
Shot blade	2	4	5	2	4	5	2-3	4	5
Flowering	4	4-6	7	4	4-6	7	4	4-6	7
Percentage of stems not infested									
Time of exposure to infestation—weeks after first exposure—									
2	55	84	100	60	63	99	48	45	100
4	53	32	37	42	37	32	32	24	39
6	81	56	76	57	69	47	38	64	75
8	94	99	98	92	99	94	99	100	99
Percentage of infested stems with oviposition scars									
2	1	6	—	1	2	—	30	18	—
4	1	0	0	1	1	1	2	8	68
6	4	1	1	2	0	0	2	1	15
8	10	—	11	0	0	5	—	—	—
Percentage of tunnelled stems not cut									
2	12	17	—	12	18	—	27	23	—
4	40	39	63	51	48	58	59	81	78
6	83	96	64	90	91	83	99	100	89
8	45	—	100	45	100	95	—	—	—

### *Experiments in the Greenhouse*

Duplicate tests were made on Rescue wheat seeded in the greenhouse in January and exposed to sawfly infestation each week after the beginning of stem elongation. Details of the methods used for greenhouse experiments on sawfly resistance have been described previously (6).

### *Collection and Analysis of Data*

In each of the three types of test all the plants under each cage were dug up at maturity. The stubs were counted, and the uncut stems were split with a razor blade and classified as (a) not infested, (b) infested stems with oviposition scars, i.e., stems containing a dead egg or a dead first-instar larva that had not tunnelled, or (c) containing a larva that had tunnelled but died before it cut the stem. The last category includes all first-instar larvae that produced tunnels visible to the naked eye. The details and limitations of this procedure have been described (5). The data are presented as percentages except in Tables 2 and 6, in which the inverse sine transformation was used in the analysis of variance.

## RESULTS

The results of the three types of experiment will be considered in connection with each of the three measures of resistance, percentage of stems not infested, percentage of infested stems with oviposition scars, and percentage of tunnelled stems not cut. In each case the data will be considered in terms of two questions—the effect of stage of development of the plant on resistance and the effect of environment on the resistance of the plant.

### *Percentage of Stems not Infested*

Table 2 summarizes the analysis of variance of the data on the percentage of stems not infested when Thatcher, Red Bobs, and Rescue plants that were sown on a single date were exposed to infestation at various times. The data in this table show that the percentages of stems not infested was low from a time shortly before shot blade to a time soon after flowering. The data in Table 3 suggest that a similar condition existed in H46146, H4191, Golden Ball, and Melanopus.

In the experiments conducted in 1952 and summarized in Table 4 it was observed that, when exposure to infestation was made on August 8, the plants that were beginning to ripen or were almost ripe showed a very high percentage of stems not infested when compared with plants that were in shot blade or had just finished flowering on that date.

Comparable data for the experiment done in 1953 (Table 5) show that the late-seeded plants had a high percentage of stems not infested when the plants were exposed to infestation at Week 2.

A summary of the analysis of variance of the data from experiments conducted in the greenhouse in the winter-time is presented in Table 6. These results show that there was a decrease in the percentage of stems not infested up to the time of shot blade.

The results of the 4 years of experiments with Rescue grown in the field are summarized in Figure 1. The data suggest that Rescue was most

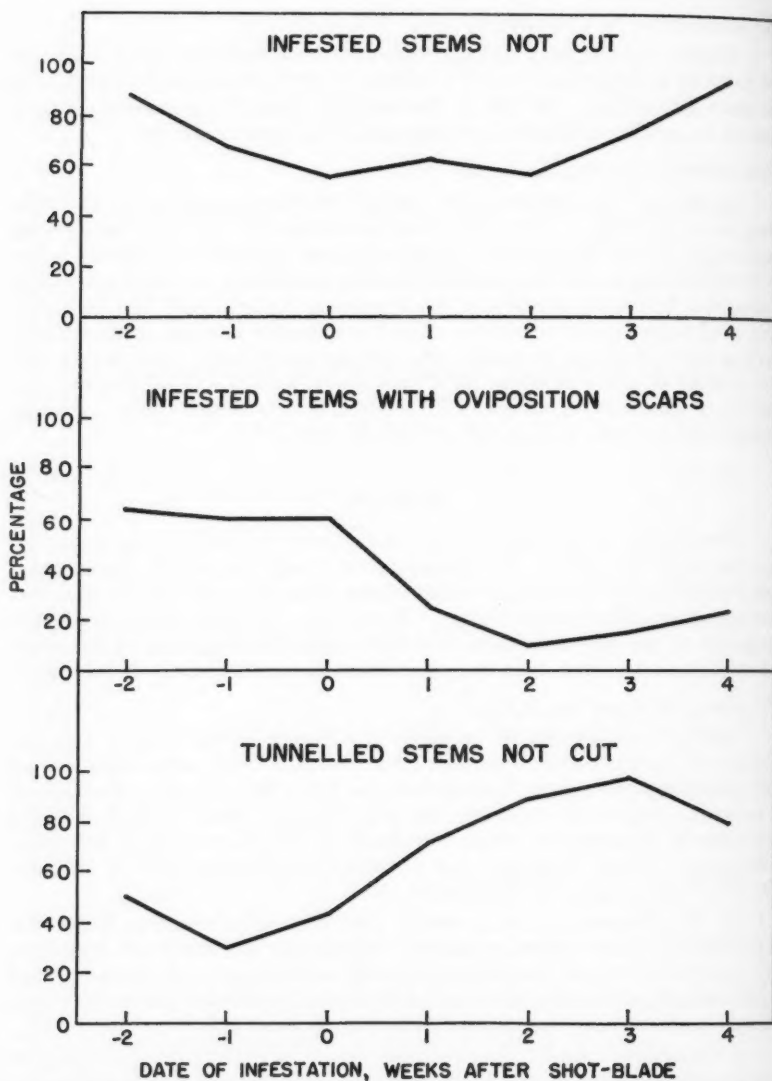


Figure 1. Resistance of Rescue wheat exposed to infestation by wheat stem sawfly at different stages of development.

heavily infested during the period from shortly before shot blade to soon after flowering. Earlier workers had observed (1, 2, 4, 8) that the boot stage was the most favourable for oviposition. Similar curves can be drawn for the data on Thatcher and Red Bobs. In spite of the environmental differences from year to year the data from each year yield somewhat

similar curves. This suggests that the shape of the curve is controlled by factors other than the environment. The 1952 data (Table 4) for plants in different stages of development on the date of exposure to infestation agree with those in Figure 1 in that they show that older plants were infested less. The 1953 results (Table 5) show that very young plants were not infested as much as older ones. The data from the greenhouse experiments show a decrease in percentage of stems not infested prior to shot blade. The results of these three types of experiment support the concept that the changes in the percentage of stems not infested that occur as the plants grow older are caused by changes in the developmental stage of the plants at the time of exposure to infestation.

The data also give some information on the effect of environment on the resistance of the plants to infestation. The analyses of variance summarized in Tables 2 and 6 show a significant interaction between weeks and years for Rescue and Thatcher. This means that the finer details of the pattern of changes in the percentage of stems not infested as the plant aged altered from year to year. The alterations are probably caused by environmental changes. However, the experiments gave no information as to whether the environment acts chiefly on the insect, chiefly on the plant, or on both insect and plant. Environmental factors are known to affect the egg laying of the insect (3).

#### *Percentage of Infested Stems with Oviposition Scars*

A summary of the analyses of variance for the data on Thatcher, Red Bobs, and Rescue sown on a single date is presented in Table 2. In the cases of Thatcher and Red Bobs there were no significant differences between the percentages of infested stems with oviposition scars when the plants were exposed to infestation on different dates. This was not true for Rescue. During the period from shot blade to flowering the percentage of infested Rescue stems with oviposition scars decreased rapidly with increasing age of plants at time of exposure to infestation. A similar decline in resistance appeared to occur in H46146, H4191, Golden Ball, and Melanopus (Table 3).

The experiments with different dates of seeding in 1952 (Table 4) showed that the percentage of infested stems with oviposition scars in Thatcher and Red Bobs was always low—below 15 per cent in all but one case. In Rescue the percentage of infested stems with oviposition scars was higher in the plants exposed to infestation at the shot-blade or boot stage than in plants exposed after flowering.

Six per cent was the upper limit for all except two of the values obtained in the 1953 experiment (Table 5) on the percentage of infested stems with oviposition scars in Thatcher and Red Bobs plants seeded on different dates and exposed to infestation at different times. In Rescue, however, this type of resistance was higher in all the young plants (second week for plants seeded on May 13 and 27, and fourth week for plants seeded on June 16) than in the older plants.

The data show that, regardless of the stage of development, the percentage of infested stems with oviposition scars was always low in Thatcher and Red Bobs. The stage of development of Thatcher and Red Bobs at

TABLE 6.—SUMMARY OF THE ANALYSIS OF VARIANCE OF DATA ON THE EFFECT OF TIME OF EXPOSURE TO INFESTATION BY WHEAT STEM SAWFLY ON THE RESISTANCE OF RESCUE WHEAT SEEDED IN JANUARY OF TWO DIFFERENT YEARS IN THE GREENHOUSE

Measurement	Average of transformed percentage values						L.S.D.	Level significance (%)		
	Date of exposure to infestation, weeks from shot blade							Weeks	Years	Weeks x years
	-2	-1	0	+1	+2	+3				
Percentage stems not infested	92	59	36	32	35	35	17	1	—	1
Percentage of infested stems with oviposition scars	—	24	19	15	19	16	—	—	—	—
Percentage tunnelled stems not cut	—	46	38	42	65	85	9	1	5	—

the time of exposure to infestation did not appear to have any effect on the resulting percentage of infested stems with oviposition scars. The results for the 4 years of experiments with Rescue are summarized in Figure 1. Examination of the data for each of the 4 years reveals a similar pattern. The experiments with Rescue sown on different dates in 1952 and 1953 indicated that young plants usually showed a higher percentage of infested stems with oviposition scars than older plants. Rescue plants grown in the greenhouse in winter-time reacted much like Thatcher and Red Bobs grown in the summer-time. These results support the conclusion that, in a resistant variety like Rescue, the percentage of infested stems with oviposition scars depends on the stage of development of the plant at the time of exposure to infestation provided that the environment is suitable for the expression of this characteristic.

That environment had an effect on the percentage of infested stems with oviposition scars is indicated by the data in Tables 2 and 6. Significant differences (1 per cent level) between years were found for both Thatcher and Rescue plants sown on a single date of seeding. The absence of any appreciable resistance to the development of eggs shown by Rescue grown in the greenhouse is also evidence for the effect of environment on this type of resistance.

The author has noticed that those varieties in which young plants show relatively high percentages of stems with oviposition scars developed tough scar tissue around unhatched eggs and dead young larvae. Roemhild (7) observed this production of brownish lignified scar tissue in Rescue and Golden Ball but not in Thatcher. These observations suggest that the presence of the egg or insertion of the ovipositor disturbs the lignin metabolism of those varieties that show this response. Lignin deposition in wheat occurs mostly during the period when the plants are heading out (11). Apparently, little new lignin is formed in older plants. Thus the period of development of most intense lignin deposition coincides with the period when sensitive wheat varieties show development of oviposition scars. It has also been observed that Red Kidney bean plants grown under high light intensities and long light exposures (9) contain more lignin. If wheat responds in the same way, then low lignin production in plants grown in the greenhouse in the winter may be associated with the failure to produce lignified oviposition scars under these conditions. Lignin is thought to be made in plants from the polymerization of phenolic compounds that have been oxidized by peroxidase (10). The formation of toxic reactive oxidation products in a reaction of this sort is quite probable. It is, therefore, possible that the presence of the egg or insertion of the ovipositor may derange the lignin metabolism of some wheat varieties in such a way that toxins that poison the developing egg or young first-instar larva are produced.

#### *Percentage of Tunnelled Stems Not Cut*

A summary of the analysis of variance of the data for Thatcher, Red Bobs, and Rescue sown on a single date and exposed to infestation at various times is presented in Table 2. It shows that the percentage of tunnelled stems not cut increased rapidly after shot blade. One year's data suggest that a similar situation existed in H46146, H4191, Golden Ball, and Melanopus (Table 3).



The data from the 1952 experiment (Table 4) with various dates of seeding show the same general trend for Thatcher, Red Bobs, and Rescue. In the 1953 experiment the results (Table 4) followed the same general trend. However, if single dates of exposure to infestation are considered, the data do not support the hypothesis that the increase in percentage of tunnelled stems not cut was caused by the stage of development of the plant. One feature of the data is quite surprising. This is the high percentage of tunnelled stems not cut in the Thatcher and Red Bobs seeded on June 16 and exposed to infestation just before they reached the shot-blade stage. High resistance previously encountered in these two varieties on irrigated land (6) was probably the result of environmental influences.

The experiment with Rescue wheat grown in the greenhouse also showed a significant increase in the percentage of tunnelled stems not cut when the older plants were exposed to infestation (Table 6).

The results of 4 years of experiments with Rescue grown in the field are summarized in Figure 1. For plants exposed to infestation after they had passed the shot blade stage the summary indicates that the percentage of tunnelled stems not cut in Rescue increased rapidly as the plant aged. The hypothesis that this effect was caused by the ageing of the plant was supported by the 1952 experiments with plants seeded on different dates and also by the experiments with Rescue wheat grown in the greenhouse in the winter-time. The data from the June 15 seedings of the 1953 experiment with Thatcher and Red Bobs indicated that environmental factors may have had an important effect on the percentage of tunnelled stems not cut.

There are at least two possible interpretations of the observed increase in the percentage of tunnelled stems not cut that occurred in plants exposed to infestation at progressively later stages of development after shot blade. These interpretations are: (a) insufficient time for the larvae to complete development in plants exposed to infestation after they have passed the shot blade stage, and (b) a progressive increase in real resistance of the plants to the insects as the plants approach maturity. The data presented do not permit a definite decision to be made between these alternatives. However, if the data for the 1953 experiments with a single date of seeding (Table 3) are examined, it will be noted that in Thatcher and Red Bobs there was no appreciable increase in the percentage of stems not cut until they were exposed to infestation at a time later than 1 week after shot blade. In the case of Golden Ball the plants reached the flowering stage before this phenomenon appeared whereas H4191 plants exposed to infestation at shot blade were already showing this increase in the percentage of tunnelled stems not cut. Thatcher and Red Bobs matured at least 2 weeks earlier than H4191 and Golden Ball. If the only cause for the increase in the percentage of tunnelled stems not cut in plants exposed to infestation at progressively later stages of development after shot blade were insufficient time for larval development, then it would be expected that this phenomenon would have appeared about 2 weeks later in both H4191 and Golden Ball than it did in Thatcher and Red Bobs. This, apparently, was not the case. It is possible, therefore, that the older plants have more resistance to the larvae than younger plants. If so, then it is likely that older wheat stems have a lower nutritional value for sawfly larvae than young wheat stems.



### DISCUSSION

In the preceding section a presentation has been made of the evidence that supports the hypothesis that changes occur in the resistance of wheat plants exposed to infestation by sawflies at progressively later stages of development. For Rescue wheat these changes are summarized in Figure 1. Furthermore it has been shown that environmental conditions affect this general pattern of changes in resistance in the plants. Under field conditions it has been observed that the natural time of the sawfly flight does not always occur when the wheat is in the same stage of development. The consequences of these observations will now be considered.

The results of the experiments on the percentage of stems not infested help to explain some of the results of Platt and Farstad (4), who made the following statements: "Statistically significant varietal differences in percentage infestation were established at each nursery, thus showing that certain varieties were infested to a greater degree than others. . . . No single variety was significantly lower at all stations. . . . When these wheats were caged in 1942 the sawflies oviposited freely, infesting them 100%. . . . The results show that none of the varieties tested had any particular resistance to oviposition, but they also show that some varieties escape infestation in varietal test plots." An examination of the data in Table 3 suggests that for a single date of seeding the ranking of varieties with respect to the percentage of stems not infested will depend on the stage of development of the plants at the time of the sawfly flight. These results make it easy to reconcile the significant differences between varieties grown at a single location with the inconsistency of varietal reaction at different stations.

If the percentage of total stems not cut is calculated for each of the varieties in the single date of seeding test for 1953 on the assumption that the duration of the sawfly flight is 4 weeks, it will be found that, in comparison with the bread wheat varieties, Golden Ball is relatively resistant if the flight is early but that it is relatively susceptible if the flight is late. Since the peak of the flight usually occurs when the wheat plants are nearly in the boot stage, it is apparent that small changes in its synchronization with the development of the wheat plants may cause supposedly resistant varieties to become susceptible. Furthermore the data show that changes in synchronization may shift the principal mechanism of resistance of the plant from the oviposition scar type of response to that form of resistance that results in the death of older larvae.

The results indicate that the resistance of a given variety of wheat to sawfly attack depends not only on the environment in which the plant is grown but also on the stage of development of the plant at the time of infestation by sawflies.

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# MANAGEMENT OF MEDIUM RED CLOVER FOR SEED AND HAY PRODUCTION<sup>1</sup>

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## ABSTRACT

Higher seed yields of Redon red clover were produced from the aftermath following an early hay crop than from the first crop itself. Red clover-timothy mixtures produced more hay in June and more seed from the aftermath than did pure stands of red clover or red clover-bromegrass mixtures. Seeding rates of 3, 6, 9 and 12 lb. per acre had no effect on red clover seed yield but did affect hay yield. With 2 lb. of timothy, no increase in hay yield was obtained with red clover seeding rates above 6 lb. per acre.

Red clover alone produced more seed per acre than red clover-timothy or red clover-brome mixtures when seed was harvested from the first crop. First-crop seed was higher in seed weight than second-crop seed and this was reflected in increased early seedling vigour.

## INTRODUCTION

Medium red clover is an important short-term forage legume in Ontario. Recently new varieties have been developed which are more persistent, higher yielding and more resistant to diseases than the common types of this legume. Following the development of these new varieties, there is need for information on crop practices which will assure high seed yields and rapid seed increase. The purpose of this study was to compare the seed yields and seed quality of Redon red clover produced 1) from first crop (July) and second crop (September); 2) alone and in simple mixtures, and 3) from different seeding rates.

In Illinois (5), Michigan (2) and Eastern Canada (1), the first crop generally has been cut for hay and the seed crop harvested from the aftermath. Workers in these areas reported higher seed yields using this system of management than when seed was harvested from the first crop. Megee *et al.* (6), however, found that in some years the seed yields were as high from the first crop as from the second. Harvesting seed from the second crop may result in lower seed quality as indicated by the results of Williams (7). He found that heads flowering during the latter part of August and the first week of September produced lighter seed than those flowering early in the season. Husenkveld (5) reported that usually red clover seed has been harvested from pure stands in Illinois. Everley *et al.* (3), however, reported that seed of this legume has been harvested from red clover-timothy mixtures in Indiana. The latter workers warned that only small amounts of timothy should be used for best seed yields of the clover.

It was found by Hollowell and Husenkveld (4) that hay yield was progressively increased as the seeding rate of red clover was increased from 5 through 10 pounds per acre. No increase in yield was obtained when the seeding rate was increased above 10 pounds.

<sup>1</sup> Condensation of a M.S.A. thesis submitted to the Graduate School, Ontario Agricultural College, by the senior author.

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TABLE 1.—SEED YIELDS OF RED CLOVER GROWN ALONE, WITH TWO GRASSES, AT FOUR SEEDING RATES AND HARVESTED AS FIRST- AND SECOND-CROP SEED FROM TWO SEEDINGS AT GUELPH

Rate of seeding red clover lb./ac.	First-crop seed (lb./acre)						Second-crop seed (lb./acre)						Seeding rate average lb./acre		
	Red clover alone		Red clover and timothy		Red clover and brome-grass		Red clover alone		Red clover and timothy		Red clover and brome-grass		First crop	Second crop	Av.
	1951 <sup>1</sup>	1952 <sup>1</sup>	1951 <sup>1</sup>	1952 <sup>1</sup>	1951 <sup>1</sup>	1952 <sup>1</sup>	1951 <sup>1</sup>	1952 <sup>1</sup>	1951 <sup>1</sup>	1952 <sup>1</sup>	1951 <sup>1</sup>	1952 <sup>1</sup>			
3	200	130	175	100	164	101	224	105	270	165	154	114	145	172	158 <sup>*</sup>
6	194	150	152	103	177	95	233	138	240	162	143	73	145	165	155
9	165	152	129	82	123	98	274	122	293	187	187	106	125	195	160
12	150	81	135	68	143	83	228	121	324	161	161	98	110	182	146
Mean	178	128	148	88	152	94	240	122	282	169	161	98			
2-year average	* 153		118		123		181		226		130		131	178	155

\* Duncan's multiple range test  $p=0.05$ . Means not underscored by the same line are significantly different.  
<sup>1</sup> Year of harvest.

### MATERIALS AND METHODS

Two trials were conducted from 1950 to 1952 at the Ontario Agricultural College, Guelph, using Redon medium red clover, a variety which flowers about 5 days later than common Ontario-grown, double-cut red clover. In each test, Redon was seeded at rates of 3, 6, 9 and 12 pounds per acre in pure stands, in mixtures with 2 pounds of Medon timothy and in mixtures with 8 pounds of Canadian brome grass. These were harvested under two managements. The first management system allowed the crop to produce seed from the first growth (first-crop). Under the second management system a crop of hay was removed at the quarter-bloom stage and the aftermath allowed to produce seed (second-crop). These treatments were included in a randomized complete block design with four replications.

In May of each crop year, red clover plant stand counts were made to determine the plant population at each seeding rate which produced the first-crop seed. Similarly, counts were made in the aftermath in July to establish the plant population that produced second-crop seed.

Three attributes of seed quality were assessed: seed weight, seed viability, and early seedling vigour. Seed weight determinations were based on triplicate samples of 100 seeds from each plot. One hundred seeds of each treatment were then planted in vermiculite in clay pots at a depth of 0.25 inches in the greenhouse in a randomized complete block design with four replications. Per cent emergence and vigour of the seedlings were recorded 10 days after emergence. Prior to analysis these data were transformed using the angular transformation. Duncan's multiple range test was used to determine differences among treatment means in seed and hay yield and seed quality of both tests and in the analyses which combined the two tests. Only differences among the combined treatment means are shown as these differences were found to be consistent in each test.

### EXPERIMENTAL RESULTS AND DISCUSSION

#### *Seed Yield*

No differences in seed yield were obtained among the seeding rates used regardless of whether seed was harvested from the first growth or from the aftermath (Table 1). On the other hand, first or second cut had a pronounced effect on seed yield. Mixture also was important in determining the level of seed yield. The heaviest volume of seed was produced from the aftermath harvest of red clover and timothy.

A mixture x management interaction was obtained. When red clover was seeded alone the first crop seed yields were higher than those of the second. When timothy was added to the mixture the second crop outyielded the first. In the red clover and brome grass mixture no difference was found between the yields from the first and second crop. This mixture was the lowest yielding of the three under test.

TABLE 2.—HAY YIELDS<sup>2</sup> OF RED CLOVER GROWN ALONE AND WITH TWO GRASSES AND AT FOUR SEEDING RATES AT GUELPH

Rate of seeding red clover lb./ac.	Red clover alone		Red clover and brome grass		Red clover and timothy		Seeding rate average tons/acre
	1951 <sup>1</sup>	1952 <sup>1</sup>	1951 <sup>1</sup>	1952 <sup>1</sup>	1951 <sup>1</sup>	1952 <sup>1</sup>	
3	2.13	1.29	1.98	1.96	2.45	1.86	1.95 <sup>*</sup>
6	2.22	1.86	2.42	1.85	2.72	2.12	2.20
9	2.52	1.87	2.23	2.08	2.65	2.07	2.24
12	2.79	1.90	2.46	1.89	2.85	2.44	2.39
Mean	2.42	1.73	2.27	1.92	2.67	2.13	
2-year average	2.07		2.11		2.39		2.19

\* Duncan's multiple range test  $p=0.05$ . Means not underscored by the same line are significantly different.

<sup>1</sup> Year of harvest.

<sup>2</sup> Tons 15 per cent forage per acre in June.

### Hay Yields

In contrast to seed yields, hay yields were affected by seeding rate (Table 2). Here forage yields were increased when the seeding rate of red clover was raised from 3 to 6 pounds per acre but not beyond this rate.

In both years the hay yields obtained from clover grown alone or with brome grass were similar but lower than those harvested from the timothy-clover mixture.

It would appear from these results that the management practices required for highest production of red clover seed are those which have been found by Bird (3) to be commonly in use in Eastern Canada; that is, an early hay crop followed by an aftermath seed crop and the use of a red clover-timothy mixture. In addition, under this system of management a seed producer should choose a rate of seeding which will result in the highest returns of forage, knowing that the seeding rate used will not affect seed yield from the aftermath. The choice of any one of the 6, 9 or 12 pounds rates of red clover per acre as were used in these tests would result in the highest forage yield.

On the other hand, where seed is to be taken from the first growth, the results indicate that no grass should be included and that a wide range of seeding rates could be used without affecting seed yield.

### Plant Population and Competition

The range in seeding rates from 3 to 9 pounds per acre resulted in a corresponding range in established plants in both tests (Table 3). In view of this relationship, and since seeding rates had no influence on seed

TABLE 3.—RED CLOVER PLANT POPULATION PER SQUARE FOOT THAT PRODUCED FIRST-CROP HAY OR SEED AND SECOND-CROP SEED AT GUELPH

Rate of seedling red clover lb./ac.	First-crop hay or seed						Second-crop seed						Seeding rate average		
	Red clover alone			Red clover and brome-grass			Red clover and timothy			Red clover alone			Red clover and brome-grass		
	1951 <sup>1</sup>		1952 <sup>1</sup>	1951 <sup>1</sup>		1952 <sup>1</sup>	1951 <sup>1</sup>		1952 <sup>1</sup>	1951 <sup>1</sup>		1952 <sup>1</sup>	1951 <sup>1</sup>		1952 <sup>1</sup>
	1951 <sup>1</sup>	1952 <sup>1</sup>		1951 <sup>1</sup>	1952 <sup>1</sup>		1951 <sup>1</sup>	1952 <sup>1</sup>		1951 <sup>1</sup>	1952 <sup>1</sup>		1951 <sup>1</sup>	1952 <sup>1</sup>	
3	3.0	5.3	3.1	5.1	3.0	5.6	2.4	4.1	2.2	5.1	1.9	5.1	4.2	3.5	3.9 <sup>1</sup>
6	4.0	8.6	3.6	9.3	4.5	8.7	2.8	8.4	2.5	6.4	2.0	7.1	6.5	4.9	5.7 <sup>1</sup>
9	5.5	13.7	5.7	13.8	5.3	10.6	2.8	9.4	2.8	9.4	2.7	6.7	9.1	5.7	7.4 <sup>1</sup>
12	7.2	17.8	6.2	12.8	6.7	11.8	3.1	12.7	3.2	8.9	2.1	6.3	10.4	6.1	8.3 <sup>1</sup>
Mean	4.9	11.4	4.7	10.3	4.9	9.2	2.8	8.7	2.7	7.5	2.2	6.3			
2-year average	* 8.2		7.5		7.1		* 5.8		5.1		4.3		7.6	5.1	6.3

\* Duncan's multiple range test  $p=0.05$ . Means not underscored by the same line are significantly different.<sup>1</sup> Year of harvest.



TABLE 4.—THE 100-SEED WEIGHT OF FIRST-CROP RED CLOVER SEED FROM PURE STANDS AND MIXTURES AND THE EMERGENCE AND VIGOUR OF SEEDLINGS AT 10 DAYS FROM SEEDING AT A DEPTH OF 0.25 INCH IN VERMICULITE

	First-crop seed			Second-crop seed			Average	
	Red clover alone	Red clover and timothy	Red clover and bromegrass	Red clover alone	Red clover and timothy	Red clover and bromegrass	First crop	Second crop
100-seed weight (mgm.)	189	179	176	148	144	134	181	142
Seedling characteristics— Per cent emergence	64	66	69	63	61	64	66	63
Early vigour <sup>1</sup>	3.3	3.3	3.3	5.0	5.0	5.0	3.3	5.0

<sup>1</sup> 1 most vigorous; 10 least vigorous.

\* Duncan's multiple range test  $p=0.05$ . Means not underscored by the same line are significantly different.



production, it follows that plant population did not affect seed yield. Adding support to this was the fact that seed was produced from these tests under two widely different climatic conditions and on different levels and ranges of plant stands but no relationship between stand and yield was obtained (1951,  $r=0.43$ ; 1952,  $r=0.20$ , both values non-significant). In the first experiment seeded in a dry year unfavourable to establishment, a good range but moderate level of red clover plants (3.0 to 6.7 plants per square foot) resulted from the seeding rates, and in the second, a moist year favourable to establishment, a high number and a wide range (5.3 to 14.1) of plants were established.

The presence of a grass appeared to have a more depressive effect upon seed production than red clover plant population. For although timothy had reduced the number of red clover plants in the first growth below that where brome grass had been added or that where red clover was seeded alone, the yields of seed from both mixtures were similar and lower than those from the pure stands of this legume. The presence of timothy (30 per cent) and brome grass (28 per cent) with their tall seed stalks may have restricted the activities of pollinating insects.

Similarly with the aftermath seed crop the movement of these insects may have been impeded causing a reduction in seed yield in the red clover-brome grass mixture as a result of the amount of foliage (20 per cent) and numerous seed stalks produced by the brome grass. In contrast no such barrier was presented to the insects in the red clover-timothy mixture as the aftermath recovery of timothy was low.

#### *Seed Quality*

Seed harvested from the aftermath was lower in unit weight than that from unclipped material (Table 4). These findings were consistent with those of Williams (7) who found that seed harvested during the latter part of August or early September was lower in seed weight than the seed harvested early. This difference in seed weight was reflected in seedling vigour but not in emergence when the seed was planted in vermiculite at a depth of 0.25 inches. Although under these optimum establishment conditions seedling emergence was not influenced by seed weight, differences were obtained when seed was planted in a subsequent test at 1.5 inches in vermiculite and in Haldimand clay (Table 5). Here the heavier seed of the first crop established more seedlings than the lower weight seed from the aftermath crop.

TABLE 5.—PER CENT EMERGENCE AT 10 DAYS FROM SEEDING AT A DEPTH OF 1.5 INCHES

Seed from	Vermiculite	Haldimand clay
First crop	26.0	45.0
Second crop	17.6	15.6

Seed weight differences among mixtures within each seed crop, although present, were not reflected in the emergence or vigour of the seedlings produced under the optimum establishment conditions of the greenhouse test (Table 4). Hence the influence on the seed weight of red clover from competition of associated grasses appeared to be of little importance, whereas the time or period when the seed was harvested appeared to be of greater importance. More work, however, is necessary under field conditions to evaluate the agronomic importance of these differences in seedling establishment.

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# THE INFLUENCE OF THE HOST ON OVIPOSITION BY THE WHEAT STEM SAWFLY, *CEPHUS CINCTUS* NORT. (HYMENOPTERA: CEPHIDAE)<sup>1</sup>

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## ABSTRACT

Differences in host development largely determined the relative times, amounts, and locations of oviposition by the wheat stem sawfly in various hosts studied under field conditions. The difference in rates of development of varieties was the main cause of differences in infestations of the varieties. Resistance to oviposition was not a factor in the sawfly resistance of any of the test varieties.

## INTRODUCTION

The wheat stem sawfly, *Cephus cinctus* Nort., cuts a slit with its saw-like ovipositor and deposits its egg inside the stem of its host in late June and early July. The act of oviposition has been described by Criddle (2) and Ainslie (1). Concurrent studies show that sawfly-resistant hosts differ in their resistance between certain internodes and between certain locations within each internode. Other studies have shown that time of infestation may influence sex ratio (9). The purpose of this study was to determine the influence of host variety and maturity on the relative times, amounts, and locations of oviposition by the wheat stem sawfly. In this paper, oviposition is regarded as infestation, and resistance as the inherent qualities of the host plant that affect the amount of sawfly damage, measured by the percentage of stems cut by the larvae.

## METHODS AND MATERIALS

The experiments were conducted at Lethbridge, Alberta, with spring wheats in 1953, 1954, 1955, 1957, and 1958 adjacent to heavy densities of sawflies, which provided natural sources of infestation. The varieties used were the bread wheats (*Triticum aestivum* L. emend Thell.) Red Bobs, Thatcher, and Rescue, and the durum wheats (*T. durum* Desf.) Golden Ball and Stewart. In addition, P. I. 17-0924 (*T. aestivum*), which was obtained from the Entomology Research Branch, U. S. Department of Agriculture, was used. Rescue and Golden Ball are solid-stemmed and sawfly-resistant; Stewart is partially solid-stemmed and often sawfly-resistant; P. I. 17-0924 is very late, hollow-stemmed, and partially resistant; Red Bobs and Thatcher are hollow-stemmed and susceptible.

In 1953, Thatcher and Red Bobs, planted on the same date, were covered with plastic screen cages, 2 feet square and 3 feet high, during the sawfly flight period to prevent infestation. One plot, which contained both varieties, was uncaged each day during most of the flight. At the end of the day, 25 stems of each variety were split and examined for infestation. In another test, stems from alternate uncaged rows of Rescue and Red Bobs were examined at regular intervals during the sawfly flight.

<sup>1</sup> Contribution from the Entomology Section, Research Station, Lethbridge, Alta.

In 1954, stems of Red Bobs, Thatcher, Rescue, and Golden Ball, planted on each of four dates, were examined at maturity. In addition, stems from plants of Red Bobs, Rescue, Golden Ball, Stewart, and P. I. 17-0924 seeded on May 7 were split and examined at intervals shortly after infestation began.

In 1955, Red Bobs, Rescue, Stewart, and Golden Ball, planted on each of three dates, were examined at intervals for infestation and for hatching of sawfly eggs during the growing season.

In 1957, 2-foot square plots, each consisting of two rows of Red Bobs and two rows of Thatcher, were caged before the sawfly flight. The plots were exposed for 6 to 48 hours consecutively at intervals during the flight. Immediately after exposure the stems in each plot were examined for infestation. A similar test was conducted in 1958 with Thatcher alone; each plot was exposed to infestation for 12 to 24 hours and examined immediately at the end of the exposure period. All examinations were made under a dissecting microscope.

## RESULTS AND DISCUSSION

### *Selection of Internode for Oviposition*

As the internodes within a stem of certain wheat varieties may vary in their sawfly resistance, it is important to know which internodes will be infested and the factors that govern their selection. In this paper the internodes are numbered in the order in which they develop, i.e., consecutively from the base. According to Anslie (1) the sawflies usually oviposit about 1 inch above the base of the fourth internode while Criddle (3) observed that the sawflies preferred to oviposit near the top node.

To understand the influence of host stem development on oviposition it is necessary to consider how the wheat stem develops. Most of the mature stems of the varieties tested had a total of five internodes, some had four and others six. Once the nodes are formed, elongation occurs first in Internode 1, then in successively higher internodes, ending with the internode that bears the head. Growth also ends in the same order, the lower internodes reaching full length before the upper internodes. Each leaf sheath, which provides a protective tube for its internode, grows rapidly once the internode associated with it starts to elongate (10).

The nodes, which develop at the base of each leaf insertion, are separated by the intercalary growth of the internodes. The cells throughout the young internode divide. When the upper part of the internode stops elongating, the meristemic activity continues in the basal part of the internode so that the internode continues to elongate after growth in the upper part has ceased. Growth in the leaf occurs in the same manner (5, 10). Prat (12) used the mechanical resistance of stems and leaf sheaths of rye to transverse cuts to show the distribution of the growth regions. Within each internode of a stem in the boot stage, the resistance decreased from the top to the base. The resistance, which was highest in the bottom internode, decreased progressively in successively higher internodes. Similar

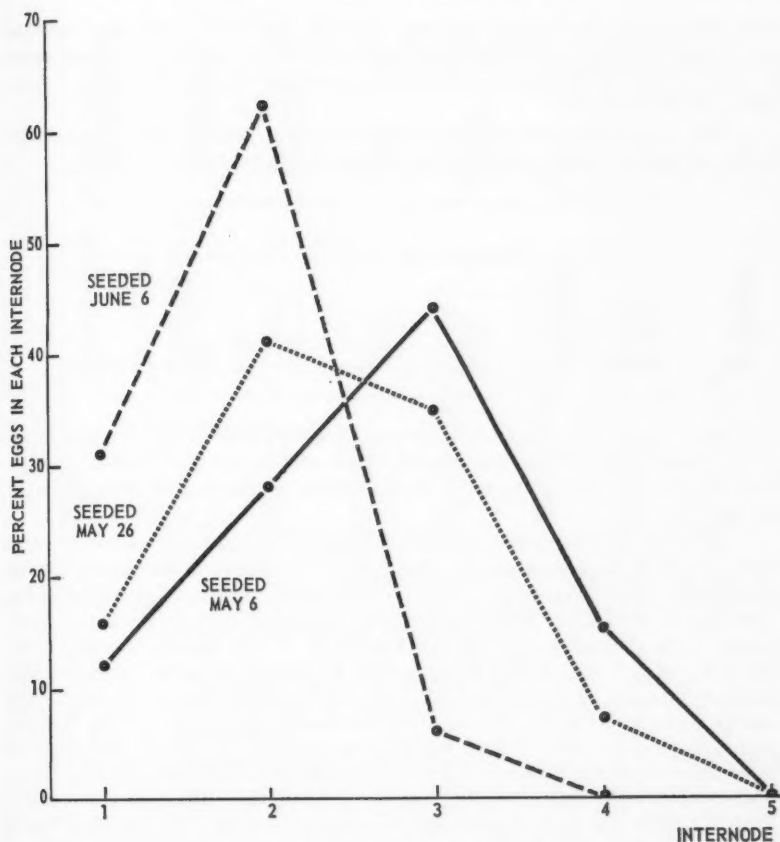


Figure 1. Distribution of sawfly eggs in Rescue wheat seeded on three dates in 1955.

results were obtained with the leaf sheaths, except that the resistance of each sheath was higher than that of the corresponding internode at the same location above the leaf insertion.

Usually a sawfly selecting an oviposition site moves first to the apex of the top leaf, then turns and moves down the stem head first to the first apparently suitable site, where she tries to insert her ovipositor. If this site is unsuitable, she will move spirally down the stem, making several attempts at oviposition in progressively lower sites until she either oviposits or flies away. Criddle (2, 3), Ainslie (1), and the present authors have not observed a sawfly oviposit more than once in the same stem unless she has flown away after the first oviposition and returned. This suggests that she may require some flight activity between ovipositions.

It is important to bear in mind then that the sawfly usually starts downward from the top of the stem and selects the first suitable oviposition

site; that the stems elongate successively from the basal internode; and that the internode that continues to elongate at the base after the upper part has stopped growing shows a decreasing resistance to transverse cuts from the apex to the base.

The percentages of the sawfly eggs that hatched in each of the internodes of Golden Ball on various dates in 1955 were:—

Date	Internode			
	1	2	3	4
July 12	6	0	0	0
July 14	16	4	3	1
July 20	80	72	60	46
July 27	100	96	88	88

The data show that oviposition generally occurs first in the bottom internode, then successively in the second, third, and fourth internodes. Similar patterns in oviposition occurred in Red Bobs, Stewart, and Rescue in the same year.

The distribution of the eggs in the stems of Rescue planted on different dates in 1955 depended on the stage of development of the stems during the sawfly flight (Figure 1). Similar data were obtained from Red Bobs, Stewart, and Golden Ball planted on the same dates. Although peak infestations generally occurred in Internode 2, the infestation decreased in Internodes 3 and 4 and increased in Internode 1 in the progressively younger plants. Internode 5 was not sufficiently elongated during the sawfly flight to be infested. In 1954 the sawfly flight was so late that the plants seeded on May 7 had matured sufficiently to cause the peaks of infestation to occur in Internode 5 of Rescue and Red Bobs and in Internode 3 of Golden Ball, Stewart, and P. I. 17-0924. The two durum and P. I. 17-0924 are later-developing varieties than the two bread wheats; it is apparent that the differences in the distributions of eggs among these varieties resulted from differences in their rates of development. Similarly, the percentages of the eggs in the bottom two internodes were higher in the two durums than in the bread wheats planted on the same dates.

Further evidence as to the effect of host development on oviposition site was obtained from the daily infestations in 1957 and 1958. The relation between the number of elongating internodes of the stems at the time of oviposition and the internodes infested was:—

No. of elongating internodes	No. of stems infested in internode					Total stems
	1	2	3	4	5	
2	22	26	—	—	—	48
3	4	191	28	—	—	217
4	1	47	121	28	—	184
5	0	0	4	22	1	25

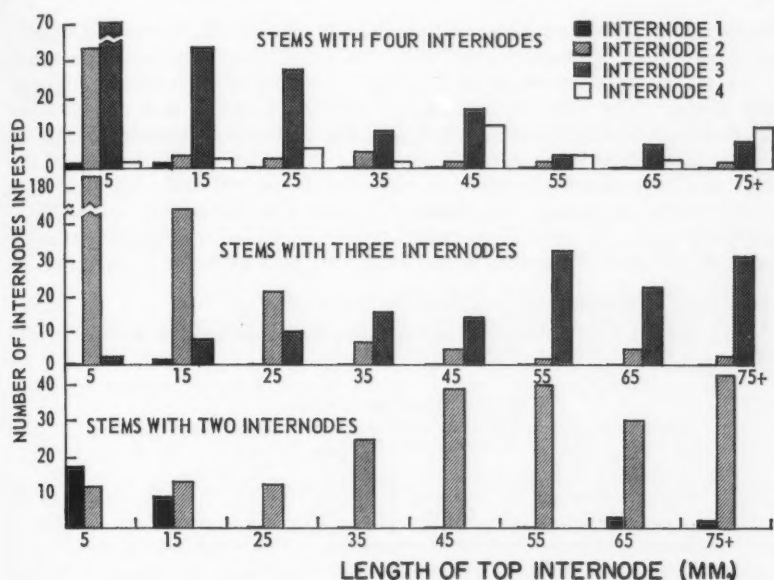


Figure 2. Effect of length of the uppermost elongating internode during the sawfly flight on selection of internode for oviposition.

Most sawflies oviposited in the second last elongating internode except in stems with only two elongating internodes; here oviposition was about equal in the two internodes.

The influence of the amount of elongation of the uppermost developing internode at the time of oviposition on oviposition site was examined. The data on internode length and internode infested were obtained from the daily exposures to sawfly in 1957 and 1958 (Figure 2). Figure 2 shows that until Internode 2 reached 10 mm., most of the sawflies oviposited in Internode 1. When Internode 2 exceeded 10 mm., it was preferred to Internode 1. In stems with three elongating internodes, Internode 2 was preferred by the sawflies until Internode 3 reached 30 mm. The preference for Internode 3 increased as its length increased over 30 mm. In stems with four elongating internodes, Internode 3 was preferred until Internode 4 reached 50 mm., but until Internode 4 exceeded 70 mm. the preference was about equal for Internodes 3 and 4. Although the data for stems with five elongating internodes were not included in the figure, as they were insufficient, they did indicate that until Internode 5 reached 80 mm. it was not infested.

As internode elongation was shown to be an important factor in selection of oviposition site, the relative amounts of elongation of adjacent internodes of differently developed stems were obtained from the 1957 and 1958 data. In most stems, Internode 3, which started to elongate when Internode 2 reached 20 mm., remained under 30 mm. until Internode 2 reached about 120 mm. Similarly, although in most stems Internode 4



started to grow when Internode 3 reached 40 mm., it did not exceed 50 mm. until Internode 3 reached about 130 mm., and when Internode 3 reached 150 mm. Internode 4 exceeded 70 mm. Great variability occurred among the stems. The relation between Internodes 4 and 5 was obscured by those stems in which Internode 4 was the ultimate internode. Thus, in stems with three internodes, Internode 2 generally received most of the eggs until it reached 120 mm.; thereafter Internode 3 had elongated sufficiently to be preferred. In stems with four internodes, Internode 3 was generally preferred until it reached 130 mm., and by the time Internode 3 reached 150 mm., Internode 4 had elongated enough to be preferred.

*Selection of Oviposition Site Within the Internode*

In 1953 the percentages of sawfly eggs laid in the upper 10 mm. of the various internodes of Red Bobs were:—

Internode			
1	2	3	4
100	94	96	100

In 1955, with the exception of Internode 3 of Red Bobs, at least 60 per cent of the eggs in each internode were laid in the top 20 mm. of each internode of four spring wheats in 1955 (Table 1).

Internode 1 in all varieties received over 50 per cent of its eggs in the top 10 mm. and almost 90 per cent in the top 20 mm. Between 75 and 94 per cent of the eggs in Internode 2 were laid in the upper 30 mm., although

TABLE 1.—PERCENTAGE OF SAWFLY EGGS LAID IN DIFFERENT LOCATIONS WITHIN THE INTERNODES OF 50 STEMS OF EACH OF FOUR SPRING WHEATS SEED ON MAY 26 AND EXAMINED ON JULY 13, 1955

Variety	Internode	No. mm. below the node						Mean length of internode
		10	20	30	40	50	50+	
Red Bobs	1	71	29	—	—	—	—	47
	2	39	21	15	12	7	7	62
	3	34	13	10	17	5	20	90
	4	60	30	10	—	—	—	35
Rescue	1	57	43	—	—	—	—	41
	2	42	23	15	12	4	4	70
	3	38	27	15	8	8	4	76
Stewart	1	91	6	2	—	—	—	47
	2	62	19	13	1	0	4	60
	3	63	0	38	—	—	—	31
Golden Ball	1	57	32	11	—	—	—	45
	2	53	23	18	4	1	—	80
	3	50	38	13	—	—	—	48

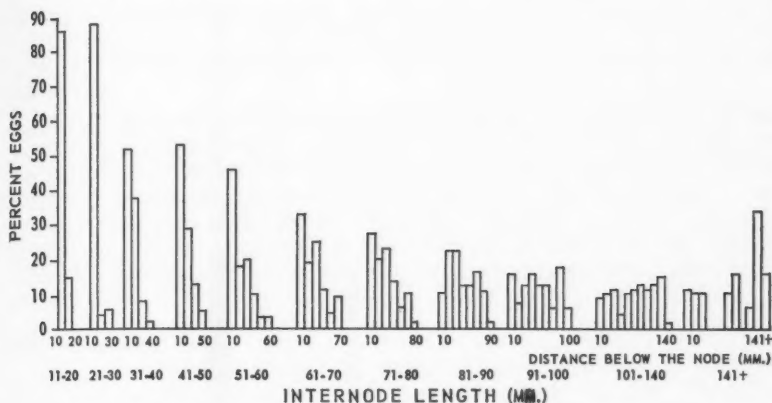


Figure 3. Effect of internode length on oviposition site within the internode.

this Internode averaged 60 to 80 mm. in length. It appears that, although a considerable length of internode is available, the sawflies mostly utilize only the upper portion for oviposition. A factor to be considered is the length to which an internode has elongated during oviposition. For example, the proportional lengths of the internodes of Rescue stems, at maturity in 1955, measured respectively from the basal internode, were 1, 1.8, 2.9, 5.7, and 6.7. The sawfly flight in 1955 ended shortly after July 13, so very few eggs were laid after that date. As Internode 3 of Rescue was only partially elongated on July 13 (Table 1), examination of these stems at a later date would suggest that oviposition was limited to a much smaller proportion of this internode than was actually the case during oviposition.

The data in 1957 and 1958 on oviposition sites were recorded within a few hours of the actual time of oviposition (Figure 3). Figure 3 shows that until the internode exceeded 50 mm. the upper 10 mm. was the preferred oviposition site. As the internode developed, the upper part became progressively less suitable for oviposition and the sawflies oviposited lower down the internode. This confirms the observations of Prat (12) that, as the internode develops, hardening occurs first at the apex and then progressively lower down the internode.

Figure 4 shows diagrammatically the locations to which the oviposition sites are limited by the growth and maturation of the stem and leaf sheaths. Only the ultimate upper portion of an internode that is just beginning to elongate is available for infestation; after the internode has elongated, the female sawflies, which appear unable to penetrate the harder mature tissue, are forced to oviposit lower down the internode. In fully elongated internodes the inner wall of the upper part appears to be quite dry while that in the lower part of the internode is still moist. Development of the sawfly egg and the internode appears to be well synchronized; by the time the inner wall of the internode has become relatively dry the eggs that were laid there when it was younger and more succulent have become sufficiently

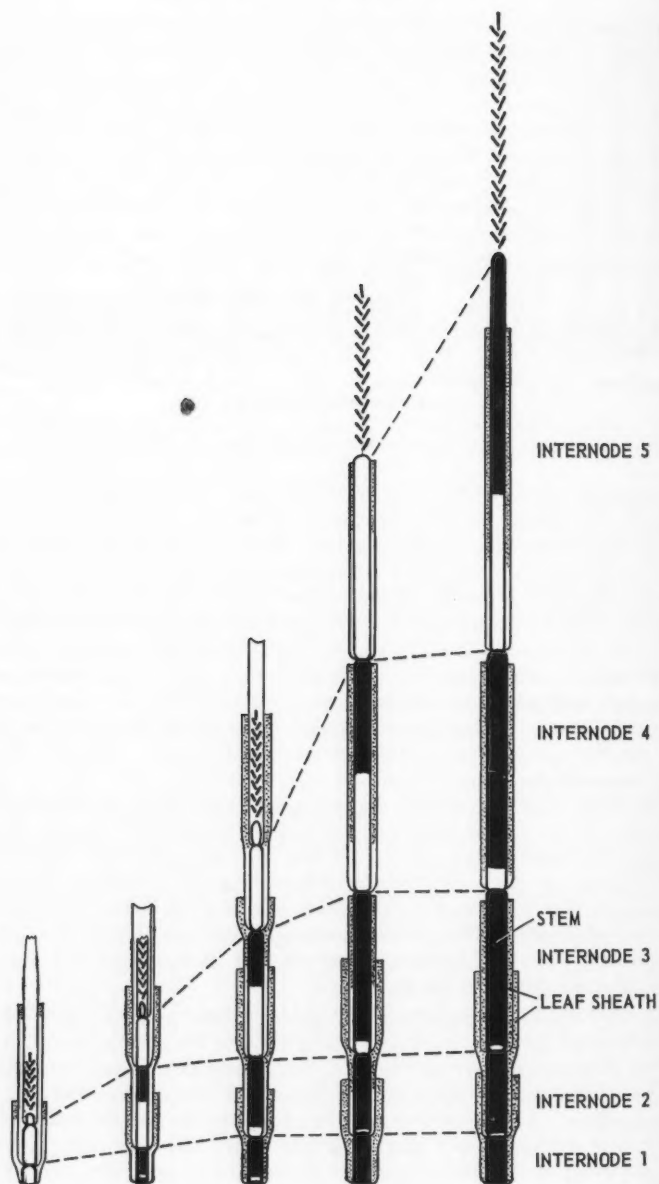


Figure 4. Favoured oviposition sites, as indicated by unshaded portions, in a wheat stem at various stages of development.

mature to require little further moisture to hatch. Fewer eggs are laid in the lower part of the internode because, by the time it is maturing at its apex, the next internode above it is elongating and will be selected for oviposition.

The leaf sheath presents an additional barrier to oviposition. In all except the top internode the female must oviposit through at least one leaf sheath, and where the sheath from a lower internode overlaps the upper internode the female must penetrate two sheaths. The upper part of the sheath, which is more mature and thus more resistant to penetration, may prevent or reduce oviposition in the lower part of the internode that it encloses; this applies particularly to the lower internodes, exclusive of Internode 1.

#### *Selection of Host Stem*

The most attractive stems for oviposition are succulent, in the boot stage, and of a suitable diameter to be readily grasped by the ovipositing female (3, 4, 6).

The requirements for a stem to be attractive for oviposition are not rigid, as females have been observed to oviposit in the leaf sheath above the head or to try to oviposit in curled leaves that are bent downward from the stem. In the laboratory they will attempt to oviposit in glass rods, dried wheat stems, and wooden rods.

The diameters, which indicate the amount of stem growth within a variety, and infestations of 50 Red Bobs and Thatcher stems that had been exposed to sawflies from the beginning of the sawfly flight recorded on June 26, 1953, were:—

Diameter (mm.) at base of third internode	No. stems	No. infested
1.90-2.20	3	0
2.21-2.50	10	3
2.51-2.80	16	8
2.81-3.10	17	11
3.11-3.40	4	4

The proportion of infested stems increased with increased stem diameter.

The number of elongating internodes, which is another measure of stem maturity, was related to oviposition preference by the daily mean infestations of stems of varying maturities on several days in 1957 and 1958. Although the tests were started about 1 week earlier in 1958 than in 1957, the plants were much more mature. The mean lengths in mm. of stems with various numbers of elongating internodes in the two years were:—

	No. of elongating internodes		
	3	4	5
June 30, 1957	85	174	238
June 22, 1958	182	260	290

TABLE 2.—THE MEAN NUMBERS OF SAWFLY EGGS LAID PER STEM IN WHEAT STEMS, WHICH ARE GROUPED ACCORDING TO NUMBER OF ELONGATING INTERNODES, ON EACH OF SEVERAL DATES IN 2 YEARS<sup>1</sup>

Date infested		No. of elongating internodes			
		2	3	4	5
1957	June 24	0.2	0.7	—	—
	June 27	0.7	0.8	3.0	—
	June 28	0.1	0.4	0.3	—
	June 29	0.3	0.6	0.8	1.4
	June 30	0.5	0.7	0.8	1.3
	July 2	0.0	0.4	0.4	0.2
	July 3	0.0	0.2	0.3	0.0
1958	June 17	0.0	0.8	0.7	—
	June 18	0.0	0.7	0.6	—
	June 21	—	0.7	0.4	0.0
	June 22	—	1.7	1.4	1.2
	June 23	—	0.0	1.3	0.9
	June 24	—	1.0	0.9	0.8
	June 25	—	1.0	0.3	0.1

<sup>1</sup> Based on at least 50 stems on each examination date

Table 2 shows that the ovipositing sawflies preferred stems with three elongating internodes to those with only two. Stems with five elongating internodes were preferred to those with three or four on June 29 and 30 but, on July 2 and 3 in 1957 and on each date in 1958, when stems with five internodes were present, those with three or four internodes were preferred. This change in preference is attributed to changes in maturity of the host stem; the stems with five internodes became less attractive as they matured. A similar change related to growth was shown by the preference between stems with three and four internodes, although the preference between these two groups of stems was not very great. On June 27, 1957, stems with four internodes were much preferred to those with three, but later the two were about equally attractive and, still later in the development of the hosts, there was a slight but consistent preference for stems with only three elongating internodes. The exception on June 23, 1958, was probably a reflection of the small sample of stems available in this category.

Although the data in Table 2 show that oviposition preference is related to the number of elongating internodes, this measurement, i.e., the number of elongating internodes, is not sufficient alone to show stem maturity. The amount of stem elongation probably provides a finer measurement. In 1953 Red Bobs and Thatcher were exposed to sawflies for 12 hours on June 20. Internode 5 had not elongated in either variety. Stems with fewer than four elongating internodes were not infested. In stems with four internodes the correlation coefficients between the total length of Internodes 3 and 4 and the number of eggs per stem for Red Bobs and for Thatcher were 0.788\* and 0.959\*\*. Thus the sawflies selected the longer stems within the range available on that date.

\*  $P=0.05$

\*\*  $P=0.01$

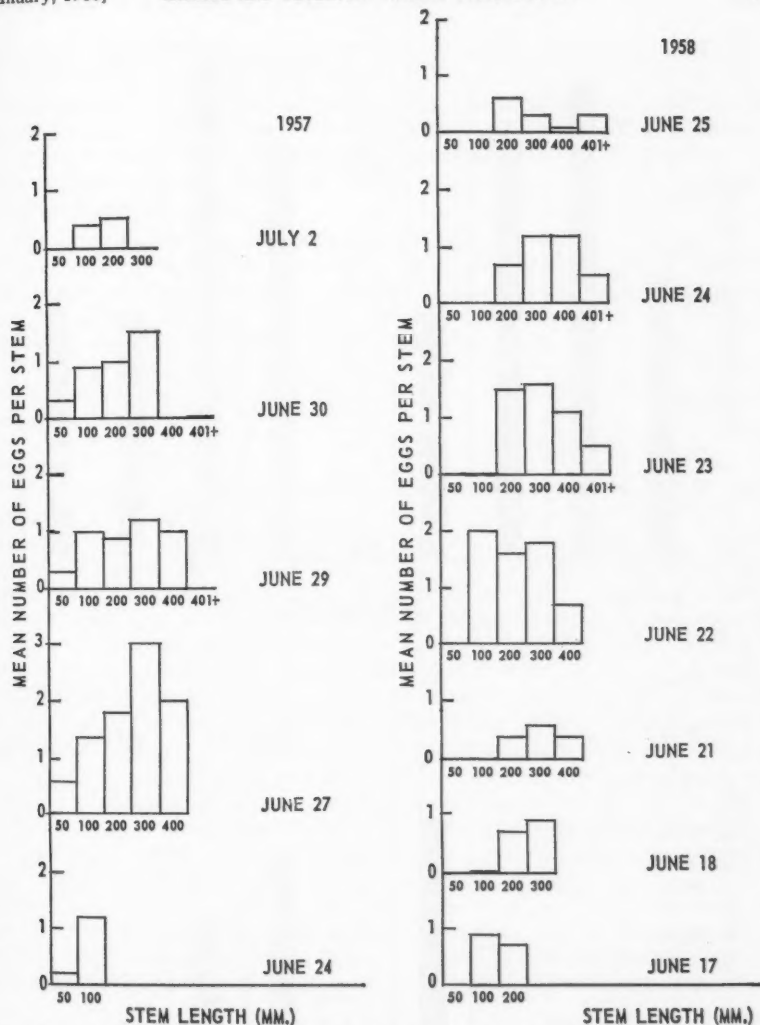


Figure 5. Effect of stem length on selection of stems for oviposition on each of several dates during the sawfly flight in 2 years.

Similar tests were conducted with daily infestations and stem measurements in 1957 and 1958. The stems were grouped according to length exclusive of the number of elongating internodes. On June 24, 1957, when the longest stems available for oviposition were 70 mm., the mean numbers of eggs per stem were:—

Stem length (mm.)						
1-10	11-20	21-30	31-40	41-50	51-60	61-70
0.0	0.0	0.1	0.2	0.3	0.7	2.0

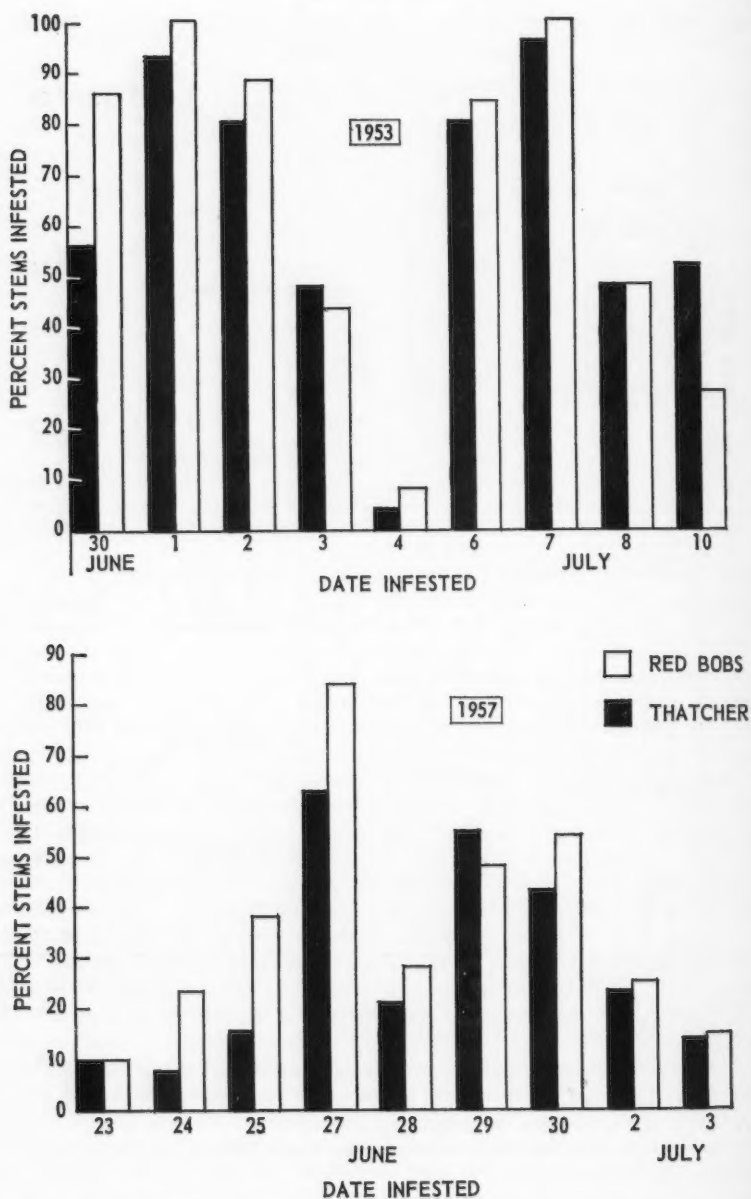


Figure 6. Infestations of two wheat varieties on each of several dates during the sawfly flight in 2 years.



The ovipositing females preferred the longer stems within this range as they did in 1953. The four later dates in 1957 gave similar results. None of the stems under 20 mm. was infested. Figure 5 shows that stems between 50 and 400 mm. were preferred for oviposition. Although host preference was not particularly strong for any of the groups of stems within this range, those between 201 and 400 mm. most frequently received the highest numbers of eggs per stem.

Thus, measurements of host maturity both by stem length and by number of elongating internodes indicate that, when stems of a wide range of maturity are available, the ovipositing sawflies prefer stems that are approximately half-grown. The boot stage occurs when the last internode is partially elongated. The preference for this stage, as mentioned by Criddle (4), would occur for a short period only when the stems were in the early part of this stage or when the youngest available stems were in the boot stage.

#### *Selection of Host Variety*

Although sawflies will infest any member of the grass family (3) as well as flax (7), they do exhibit oviposition preferences. Until 1907, sawflies selected their native hosts in preference to wheat but, since that year, when large numbers infested wheat and rye, they have oviposited as readily in cultivated plants as in their wild hosts (2). Sawflies show oviposition preferences for some varieties of barley over others, but when wheat and barley are grown together they select wheat (8). Winter wheats in the Canadian prairies are often infested lightly or not at all. However, this is escape rather than resistance, as much of the winter wheat is often too mature to be infested when the sawfly flight occurs. When winter wheat is late or the sawfly flight early, heavy infestations occur.

Infestations of various spring wheats were compared for varietal preferences. Red Bobs is often more heavily infested than Rescue and, as will be shown later, is the earlier variety. In 1953, although Rescue received 3.5 eggs per infested stem, Red Bobs averaged 7.4. In plants seeded on May 6 and 26 in 1955, Red Bobs averaged considerably more eggs per infested stem than did Rescue. The differences between these two varieties may depend at least partly on differences in their rates of development. In 1954, when many of the Red Bobs stems and few of the Rescue stems were almost fully elongated by the time of the sawfly flight, Rescue received

TABLE 3.—THE PERCENTAGES OF STEMS OF FOUR WHEATS SEEDDED ON DIFFERENT DATES THAT WERE INFESTED BY *C. cinctus* IN 1954<sup>1</sup>

Host variety	Date seeded			
	May 7	May 18	June 3	June 14
Red Bobs	83	86	93	63
Thatcher	85	84	93	41
Rescue	83	80	88	11
Golden Ball	90	97	90	19

<sup>1</sup> Based on at least 100 stems per variety for each seeding date

2.0 eggs per stem and Red Bobs 1.6. Thus varietal differences in infestations may be largely the result of the differences in the times that the varieties reach their peak of attractiveness and the coincidence of these peaks with the peak of sawfly activity. That this is an important factor is shown by a comparison of the infestations of plants within a variety seeded on different dates in the same year. The mean numbers of eggs oviposited in Red Bobs planted on May 6, 26, and June 6 in 1955 were 6.2, 5.7, and 1.5. Three other varieties gave similar results. The earliest-seeded plants were in the most suitable stage for infestation when the peak of oviposition activity occurred. In Red Bobs in 1954 (Table 3) the highest infestation occurred in the plants seeded on June 3, while those planted earlier or later received lower infestations. Similar results were obtained with Rescue and Thatcher. In that year those plants seeded earlier than June 3 had passed their peaks of attractiveness before the peak of oviposition activity, while those seeded after June 3 had not yet reached their peak of attractiveness. In Golden Ball in 1954 the peak infestation occurred in plants seeded on May 18. This agrees with the observation that Golden Ball is a later variety than the other three.

To determine whether varieties do change with growth in their relative attractiveness as hosts, comparisons were made between the infestations in Red Bobs and Thatcher on different days during the sawfly flight periods in 1953 and 1957 (Figure 6). This shows a generally consistent preference for Red Bobs that was greatest at the beginning of the flight period and decreased as the flight progressed. In 1957 the difference in infestation of the two varieties on the last 2 days of the flight was only 2 and 1 per cent, respectively. In 1953 the two varieties were equally infested. On July 8 and on July 10 the infestation in Thatcher was almost twice that in Red Bobs. The decreased differences between Red Bobs and Thatcher as they developed suggests that the major differences in the preference depended on differences in their rates of growth. By June 24, 1957, 44 per cent of the Red Bobs stems had three elongating internodes while none of the Thatcher stems had more than two elongating internodes. Six days later, 44 per cent of the Red Bobs stems had four and five elongating internodes while only 26 per cent of the Thatcher stems had reached this stage. These data suggest that because Red Bobs was more developed than Thatcher at the beginning of the flight it was preferred to Thatcher. However, as the season advanced and more of the Thatcher stems reached the preferred stage for oviposition, the differences in oviposition between the two varieties decreased. Still later in the season, more stems of Red Bobs than of Thatcher passed the preferred stage for oviposition, as on July 10, 1953, so Thatcher stems became more highly infested.

Thus the difference in growth rates of Thatcher and Red Bobs appears to account for the difference in sawfly infestations of these two varieties. Other evidence is available to show that differences in growth rates account for differences in infestations of other varieties as well as for Red Bobs and Thatcher. It was shown previously that the strongest preferences occurred within the lower range of the infestable stage, i.e., 20 and 100 mm., for the longer stems. Therefore, if varieties are planted sufficiently late that they barely reach the infestable stage at the end of the sawfly flight, distinct

TABLE 4.—SAWFLY INFESTATIONS AND MEAN LENGTHS OF INTERNODES OF SPRING WHEATS SEEDED ON DIFFERENT DATES IN 1955<sup>1</sup>

Date planted	Date examined	Host variety	Internode length (mm.)					Mean number of eggs per infested stem	Percentage of stems infested
			1	2	3	4	5		
May 6	July 6	Red Bobs	—	—	—	—	—	6.2	98
		Rescue	—	—	—	—	—	4.0	92
		Golden Ball	25	59	47	17	0	5.0	97
		Stewart	34	60	29	21	0	4.1	89
May 26	July 13	Red Bobs	47	62	90	35	0	5.7	92
		Rescue	41	70	76	0	0	2.9	90
		Golden Ball	45	80	48	0	0	3.0	97
		Stewart	47	60	31	4	0	2.8	90
June 6	July 15	Red Bobs	—	—	—	—	—	1.5	86
		Rescue	—	—	—	—	—	1.9	80
		Golden Ball	—	—	—	—	—	1.3	12
		Stewart	—	—	—	—	—	1.2	32

<sup>1</sup> Based on 50 stems of each variety on each examination date

differences in infestation should occur between varieties that differ in growth rate. Since the flight is practically ended, later infestations will not occur to mask the infestations that occurred in the early stages of development in these varieties. Infestations of the last date of seeding (June 14) in 1954 (Table 3) showed that the order in which the varieties became suitable for infestation was: Red Bobs first, then Thatcher, Golden Ball, and Rescue. In 1955 (Table 4) infestations of the last date of seeding, June 6, showed that the order in which the varieties became suitable for infestation was Red Bobs, Rescue, Stewart, and Golden Ball. Table 4 also shows, by the amount of elongation of the various internodes and, in particular, Internodes 3 and 4 on July 6 and 13, that the growth rate of the varieties followed the same order as for the infestations of these varieties planted on the last date.

The relative times of egg hatching in the varieties were compared to determine whether infestations of the varieties occurred at different times and whether this comparison agreed with the previous findings concerning the relative times that the varieties became suitable for infestation. On July 19 the percentages of sawfly eggs that hatched in Rescue planted on May 6, 26, and June 6 were 62, 49, and 8, respectively, which shows that the time of infestation follows the same order in which the host plants mature. In 1955 the percentages of eggs that hatched on July 14 in Red Bobs, Rescue, Golden Ball, and Stewart, all planted on May 6, were 18, 13, 4, and 4, respectively. These data confirm the hypothesis that these varieties become suitable for infestation at different times and that the order in which they became suitable was approximately the same as that previously indicated.

#### *Infestation as a Factor in Sawfly Resistance*

Platt and Farstad (8) found differences in the sawfly infestations of 88 varieties of spring wheat, but no one variety was significantly less infested at all stations. Two wheats, *T. dicoccum* Schrank and *T. timopheevi* Zhuk., which were usually infested to a much lesser extent than the others, were completely infested when sawflies were caged over them. They

TABLE 5.—SAWFLY INFESTATION OF VARIOUS HOSTS PLANTED ON THE SAME DATE IN 1954<sup>1</sup>

Host variety	Percentage of stems infested	No. of eggs per infested stem
Red Bobs	92	1.6
Rescue	88	2.0
Chinook	94	—
Golden Ball	97	2.2
Stewart	94	2.0
P.I. 17-0924	96	2.0
Victory (oats)	89	2.1

<sup>1</sup> Based on 100 stems of the oat variety, and 500-1300 stems of each of the wheats

concluded that none of the varieties was resistant to infestation but that some of them partially escaped infestation. Roberts (13) found differences among infestations of seven varieties tested for 3 years and he also found that none of the varieties was significantly lower each year. However, he concluded that 'infestability' was one of the varietal factors in host resistance to sawfly. The varieties tested by Roberts fell into three equally infested groups: Red Bobs and Thatcher; two durums, Golden Ball and Melanopus; and a group in which Rescue differed from two closely related lines in only 1 of the 3 years of his test. In the present paper Golden Ball was shown to be at least as susceptible to infestation as Red Bobs (Tables 3 and 4). Rescue was infested in excess of 98 per cent in 1953 when it was planted in alternate rows with Red Bobs. In 1950, when sawflies were caged over Rescue and Red Bobs together, the percentage infestations were 92 and 95. In 1952, when sawflies were caged over Rescue alone, the infestation was 93 per cent. Similarly, in 1954 and 1955 (Tables 3 and 4) the percentage of stems infested differed little between Rescue and Red Bobs.

The tests in 1954 (Table 5) included varieties that varied from susceptible to immune and yet all were heavily infested. If 'infestability' is a varietal factor in host resistance, it must reduce sawfly damage more in one variety than in another. The high infestations in the resistant varieties, even when susceptible varieties were readily available, show that 'infestability' in these varieties cannot be considered as a factor in their resistance to sawfly damage. Moreover, lowered host preference can be considered a factor in resistance only if it is strong enough to be operative when preferred varieties are not available or when adequate densities of ovipositing sawflies are present. It is apparent, therefore, that Roberts found oviposition preferences among the varieties that he tested and that, under the conditions of his test, contributed to the relative amount of sawfly damage to these varieties. However, he failed to realize that these were preferences

and that they might not be expressed when only one variety was available, as under normal field conditions, that they depended largely on the relative stages of development of these varieties during oviposition, or that they are not expressed when adequate densities of ovipositing sawflies are present. Our data strongly indicate that Roberts was in error in including 'infestability' as one of the types of varietal resistance to the wheat stem sawfly.

### CONCLUSIONS

Host development during the sawfly flight was the major factor in determining oviposition sites. The sawflies oviposited mostly in the lower internodes of the later-developing stems and in the higher internodes of earlier-developing stems. Thus the numbers of sawfly eggs were proportionally greater in the lower internodes of the durum wheats than in the lower internodes of the faster-growing bread wheats seeded on the same date. In stems with two elongating internodes at the time of oviposition both internodes were about equally infested, while in stems with more than two internodes the second last elongating internode received most of the eggs. The length of the top elongating internode strongly influenced the ovipositing females in their selection of an internode for infestation. When Internode 2 exceeded 10 mm., it was preferred to Internode 1; when Internode 3 exceeded 30 mm., it was preferred to Internode 2. Internode 3 was preferred to Internode 4 until Internode 4 reached 50 mm., but until Internode 4 reached 70 mm., it was not preferred to Internode 3.

Most eggs were laid in the top 10 mm. of internodes up to 50 mm. in length. As the internode elongated, its apex became progressively less suitable for oviposition and the sawflies were forced to oviposit farther down the internode. More eggs were not laid in the lower part of the internodes because, by the time the internode had elongated sufficiently to force the sawflies lower down, the next internode above had elongated sufficiently to be preferred for oviposition.

Maturity of stem during the flight period influenced the ovipositing females in their selection of host stems. Stems with larger diameters, and thus greater development, were preferred by the sawflies during late June. The relation between stem length and oviposition preference was also examined. Although the sawflies oviposited in stems that varied in length from 20 to over 400 mm., length of stem influenced their selection of stems within this range. When stems up to only 70 mm. were available, the longer stems were preferred by the sawflies, but when stems exceeding 400 mm. were available, the highest infestations occurred in those from 51 to 400 mm. with the peaks of infestation occurring most frequently in those between 200 and 300 mm.

Oviposition preferences were also related to the number of elongating internodes at the time of infestation. During the first part of the flight period oviposition increased progressively in stems with progressively greater numbers of elongating internodes. Later, as the stems developed, those with only three or four elongating internodes were preferred, and still later those with only three internodes were preferred.

The time of infestation varied in location within the stem, within a variety planted on different dates, and among varieties with different growth rates. Infestation occurred first in the lower internodes; then progressively later in the higher internodes within a stem; and, among stems of different maturities, first in the most mature that were still suitable for infestation and then in the progressively later stems.

Varietal differences in infestation were attributed mainly to differences in the growth rates of the varieties and not to differences in varietal resistance to oviposition; the heaviest infestations occur in a variety when its optimum stage for oviposition coincides with the peak of oviposition activity.

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# EFFECTS OF GIBBERELLIN TREATMENTS ON GERMINATION OF VARIOUS SPECIES OF WEED SEEDS

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## ABSTRACT

Nineteen species of weed seeds collected near Edmonton during 1957 and/or 1958 were tested periodically for their germination percentage with or without treatments with gibberellin. Most of the untreated samples were completely dormant under conditions of the test at harvest time and the majority of the dormant species remained so during a storage period of 6 months at  $3^{\circ}\text{C.} \pm 0.5^{\circ}$ . Gibberellin treatments of up to 500 p.p.m. in the medium, or of up to 2,000 p.p.m. as a 24-hour pre-soaking treatment, were without appreciable effect on most of the dormant species. The chemical had a significant effect in overcoming dormancy of some, but not all, samples of wild oats. Dormant seeds of wild mustard and stinkweed were very sensitive to gibberellin treatments, while hemp nettle and blue bur showed a smaller response. Differences in results between different lots of certain species emphasize the variable complexity of conditions which at a given time may or may not be responsive to gibberellin in initiating or accelerating the process of germination.

## INTRODUCTION

Dormancy and irregular germination of weed seeds are responsible for a major part of the difficulty and expense of weed control. Numerous investigations, over many years, have studied basic and practical aspects of the problem (4). The work has included extensive research on the influence of chemicals in shortening the period of dormancy of seeds of desirable and undesirable species of plants. Popular use of growth regulators as selective herbicides and for other purposes with plants during the past decade, has also provided impetus for such studies with seeds. The relatively recent availability of gibberellin has led to manifold projects involving this substance in agricultural problems (5) (6). The present paper deals with the germination response to gibberellin treatments of freshly harvested and of stored seed from various species of plants.

## MATERIALS AND METHODS

In 1957 seed gathered during the last week of September was used in a series of germination tests commencing on October 10. All of the species listed in Table 1, except wild oats, were collected from natural infestations on uncultivated disturbed areas, at Edmonton. At the time of the initial germination tests the reserves of seed were placed in storage, in stoppered flasks, at  $3^{\circ}\text{C.} \pm 0.5^{\circ}$ , for later experiments. Seed collected after June 1958 was kept at room temperature.

For the treatments the samples of seeds were soaked for 24 hours in distilled water, 100, 200, 500, 1000 and 2000 p.p.m. acid-equivalent concentrations of an aqueous solution of the potassium salt of gibberellic acid, before placing the seeds in Petri dishes on filter paper moistened with

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TABLE 1.—PERCENTAGE GERMINATION, IN THE DARK, AT 21° C., OF WEED SEEDS COLLECTED IN 1957 AND 1958

Species <sup>1</sup>	Collection	Germination %	
		Before storage	After 6 months' storage
<i>Avena fatua</i> (L.), wild oats (greenhouse samples)	Sept. 1957	25	—
	Dec. 1957	0	—
	March 1958	26	—
(field samples from plants)	July 1958	3	—
	Aug. 1958	0	40 (3 mo.)
(field samples from soil surface)	Oct. 1958	0	0 (3 mo.)
<i>Polygonum persicaria</i> (L.), lady's thumb	Sept. 1957	4	56
<i>Polygonum convolvulus</i> (L.), wild buckwheat	Sept. 1957	0	0
<i>Axyris amaranthoides</i> (L.), Russian pigweed	Sept. 1957	4	9
<i>Chenopodium album</i> (L.), lamb's quarters	Sept. 1957	0	40
<i>Amaranthus retroflexus</i> (L.), red-root pigweed	Sept. 1957	0	2
<i>Silene noctiflora</i> (L.), night-flowering catch-fly	Sept. 1957	72	96
<i>Stellaria media</i> (L.), common chickweed	Sept. 1957	16	52
<i>Neslia paniculata</i> (L.), ball mustard	Sept. 1957	0	8
<i>Sinapis arvensis</i> (L.), wild mustard	Sept. 1957	92	82
	Aug. 1958	9	—
<i>Thlaspi arvense</i> (L.), stinkweed	Sept. 1957	0	0
	July 1958	0	—
<i>Potentilla norvegica</i> (L.), rough cinquefoil	Sept. 1957	0	0
<i>Melilotus alba</i> (Desv.), sweet clover	Sept. 1957	0	40
<i>Cicuta maculata</i> (L.), water hemlock	Sept. 1957	0	0
<i>Lappula echinata</i> (Gilib.), blue bur	Sept. 1957	0	0
	July 1958	1	—
<i>Galeopsis tetrahit</i> (L.), hemp nettle	Sept. 1957	20	14
<i>Moldavica parviflora</i> (Nutt.), dragon head	Sept. 1957	0	2
	Aug. 1958	0	3 (4 mo.)
<i>Linaria vulgaris</i> (Miller), toad flax	Sept. 1957	0	0
<i>Plantago major</i> (L.), plantain	Sept. 1957	0	0

<sup>1</sup> Species are arranged in taxonomic sequence of their families.

water. The dishes were transferred to a germinator, without light, with temperature held at 21° C.  $\pm$  0.5°. Supplementary trials involved continuous exposure of the seeds to concentrations of the chemical, from 50 to 500 p.p.m., used to moisten the filter paper for the duration of the test, usually 1 week to 10 days. In a few special cases, noted later, concentrations up to 8000 p.p.m. gibberellin were employed to determine whether threshold concentrations for stimulation by this chemical might not have been reached in earlier trials.

Fifty or one hundred seeds per treatment were used in the initial tests. These were followed by similar determinations repeated at intervals of several weeks, and, whenever interesting differences were apparent, by additional experiments involving analysis of variance for four concurrent replicates of 50 or 100 seeds per treatment per replicate.

Table 1 lists the species tested and shows the percentage germination of untreated control lots within 2 weeks after their collection time and again after 6 months' storage, except where noted otherwise (Table 1).

TABLE 2.—PERCENTAGE GERMINATION FOLLOWING GIBBERELLIN TREATMENT AT DIFFERENT TIMES AFTER COLLECTION OF VARIOUS SPECIES OF WEED SEEDS

Collection	Treatment	Time after collection	Germination %		
			Check	Treated	Diff.
<i>Wild oats from greenhouse</i>					
Sept. 1957	soaked 24 hr., 1000 p.p.m.	1 wk.	25	87	62**
Dec. 1957	soaked 24 hr., 1000 p.p.m.	1 wk.	0	5	5
Mar. 1958	500 p.p.m. continuously	1 wk.	26	57	31**
<i>Wild oats from field</i>					
Aug. 1958 (from plants)	500 p.p.m. continuously	2 wk.	0	66	66**
		2 mo.	21	84	63**
		3 mo.	40	92	52**
Oct. 1958 (from soil surface)	500 p.p.m. continuously	2 wk.	0	2	2
		2 mo.	0	0	0
		3 mo.	0	0	0
	8000 p.p.m. continuously	2 mo.	0	0	0
<i>Wild mustard</i>					
Sept. 1957		2 wk.	92	—	—
	500 p.p.m. continuously	6 mo.	82	84	2
Aug. 1958	soaked 24 hr. 500 p.p.m.	2 wk.	9	89	80**
<i>Stinkweed</i>					
Sept. 1957	soaked 24 hr. 1000 p.p.m.	3 wk.	0	100	100**
	soaked 24 hr. 1000 p.p.m.	2 mo.	0	99	99**
	500 p.p.m. continuously	6 mo.	0	96	96**
July 1958	500 p.p.m. continuously	4 days	0	91	91**
<i>Blue bur</i>					
Sept. 1957	soaked 24 hr. 1000 p.p.m.	3 wk.	4	6	2
	500 p.p.m. continuously	6 mo.	0	14	14 <sup>1</sup>
July 1958	soaked 24 hr. 500 p.p.m.	3 wks.	1	16	16
<i>Hemp nettle</i>					
Sept. 1957		2 wk.	20	—	—
	soaked 24 hr. 1000 p.p.m.	10 wk.	19	44	25*
	500 p.p.m. continuously	6 mo.	14	36	22*

\*Difference significant at 5% level

\*\*Difference significant at 1% level

<sup>1</sup>Single trial

Table 2 is a representative summary of the results of the treatments excluding data from several tests with other concentrations of chemical and intermediate periods of time after seed collection that were of no additional interpretative value. Also omitted are the results for species included in Table 1, whose germination percentage was either not affected or perhaps very slightly improved by the 500 or 1000 p.p.m. treatments with gibberellin (Table 2).

#### DISCUSSION AND CONCLUSIONS

Recognizing restrictions on interpretation of results, commonly required in work of this nature, it is evident that the constant temperature used for the germination tests with these various species may not have supplied optimum conditions. The figures shown in Table 1, for percentage germination before and after cold storage, are, therefore, subject to this possible limitation. Consequently the observed effects of the gibberellin treatments upon germination might be either greater, or less, than a 'normal' response. Nevertheless some relative comparisons are instructive. In Table 1 the majority of the species that did not germinate well soon after collection apparently were maintained in that condition during the cold storage. Notable exceptions were lady's thumb, lamb's quarters, chickweed and sweet clover.

There was a striking difference between the early post-harvest germination of different collections of wild oats and of wild mustard seed, which showed no visible differences in their respective appearance at harvest time. It seems reasonable to expect that similar differences in germination might have been found for some of the other species as well, had additional material been tested.

The germination of only a few of the species was clearly stimulated by gibberellin treatments as great as 2000 p.p.m. for a 24-hour pre-soaking period in the earlier tests, or 500 p.p.m. in the medium during the germination period in the later trials. None of the concentrations, used within the ranges mentioned earlier, reduced germination of the species tested. Table 2 illustrates the positive results obtained with certain samples of wild oats, wild mustard, stinkweed, blue bur and hemp nettle.

The differences between results for collections of wild oats from greenhouse material of different plantings grown from the same seed source, and for collections from different sources in the field, emphasize the point that sometimes gibberellin induces a recognizable response, sometimes it does not, within the same species. The three lots of wild oats grown in the greenhouse were cared for in the same way during the growing period and all reached maturity; hence the differences in germinability must be mainly attributable to variation induced by seasonal fluctuations in the natural environment. The failure of wild oats that had fallen to the ground in the field to respond to gibberellin treatments at the times shown suggests similar differences, perhaps reinforced by changes occurring after the seeds were in contact with the soil. Supplementary trials with very high concentrations of the chemical (8000, 12000 p.p.m.) failed to increase the germination of such seeds. Perhaps this should be expected since, from earlier experience with wheat seeds, we would anticipate inhibition rather than stimulation from such high rates of gibberellin. Apparently an appropriate state of "ripeness" is required before gibberellin can hasten or improve the percentage germination by overcoming inhibiting substances or processes. This view is in agreement with earlier findings by Vanden Born and Corns (5), that partially after-ripened seeds of Tartary buckwheat, but not fully dormant seeds, would respond to gibberellin treatments. On the other hand, in the present studies, the dormant seeds of wild mustard and of stinkweed, another crucifer, were very sensitive to gibberellin soon after their collection from the parent plants.

The results for wild mustard of 1957 indicate that with an after-ripened sample of this seed there was no benefit from gibberellin treatment. This was also true for the night-flowering catch-fly sample. In contrast, however, almost completely dormant seeds of the 1958 lot of wild mustard exhibited an immediate response to gibberellin treatment.

With stinkweed there was an extraordinary and consistent virtually complete germination of dormant seeds after gibberellin treatments of 1000 or 500 p.p.m. (Table 2), and of 100 p.p.m. in some trials not listed in the Table. As little as 25 p.p.m. of the chemical caused about 10 per cent germination, and 50 p.p.m. induced approximately 50 per cent germination of otherwise dormant seeds, thus illustrating quantitative aspects of the reaction.

Much of the earlier work has shown promotion of germination following marked fluctuations in temperature and drying of the seeds (4, ch. 7 and 9). The prolonged dormancy of several species of weed seeds not receiving such manipulation in the current tests might be explained, at least in part, on this basis and is consistent with behaviour of several species of weeds under field conditions (1, 2). The well-known phenomenon of periodicity of germination is, of course, evidence for the absence of uniformity of physiological behaviour even within lots of seeds and is illustrative of the complexity of the problem of interpretation of dormancy.

The data with reference to the effects of gibberellin on seed germination show the need for caution about over-simplified statements concerning the role of this chemical in germination processes of seeds in general or in particular. Intensive study, under a variety of controlled environmental conditions, of the production of growth-inhibiting substances in the seeds of individual species, should help to clarify the variability in germination within and between species. Concurrent experiments involving gibberellin treatments would be desirable in efforts to understand the interrelationships responsible for variability in the stimulatory role of this chemical for seed germination. Thorough elucidation of the basic biochemical mechanisms probably constitutes a much less hopeful task.

#### ACKNOWLEDGEMENTS

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# A STUDY OF THE RELATIONSHIP BETWEEN THE AMOUNT OF BLOOM AND YIELD OF APPLES<sup>1</sup>

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## ABSTRACT

The fruit yield of apple trees per unit area of trunk cross-section is shown to be very closely related to the author's estimate of the amount of bloom. Trees with maximum bloom score produced 46.48 kilograms of fruit per 100 square cm. of trunk cross-section, and lower percentages of bloom showed a linear relationship between the amount of bloom and yield of fruit.

## INTRODUCTION

Observations of commercial orchards as well as of the orchards of the Kentville Experimental Farm have prompted the two following questions: Is there a relationship between amount of bloom carried by an apple tree and the amount of crop it produces; and, as a corollary, under normal conditions how much bloom on apple trees is needed to produce a full crop?

## REVIEW OF LITERATURE

It has been reported that a 5 per cent set of the flowers on a tree with a "snowball" bloom would give sufficient apples for a full crop (1, 3), but this is considered an average figure and would vary with the variety (1). Roberts (7) reported that the percentage set of fruit in Delicious in the Eastern and Mid-western United States and in Eastern Canada showed an inverse relation to the percentage of blossoming. The same idea was suggested by Brittain (1). These were general statements.

Variability in yield and growth of apple trees is known to be high. Hoblyn (4) reporting on variability states that it "is thus difficult of comparison, but in nearly all cases given, the coefficients are between 30 and 40 per cent of the mean and in some cases over 50 per cent. Parker and Batchelor (5) state that "the coefficient of variability of individual trees has been reported to lie within the extremely broad range from 19.66 per cent and 89.6 per cent" and Pearee (6) reports an instance of trees on free stock with 27 to 151 per cent variability during different periods.

## MATERIALS AND METHODS

The study was based on the regular annual orchard records of the Kentville Experimental Farm. This orchard, when the study began, included 450 trees set in 1940, 265 trees set in 1939, 190 trees set in 1934, approximately 800 trees set in 1930, and about 220 older trees. Varieties with fewer than 8 trees were excluded from the study. When fruit was thinned, the trees were excluded.

<sup>1</sup>Contribution No. 1029 of the Research Station, Canada Agriculture, Kentville, N.S.

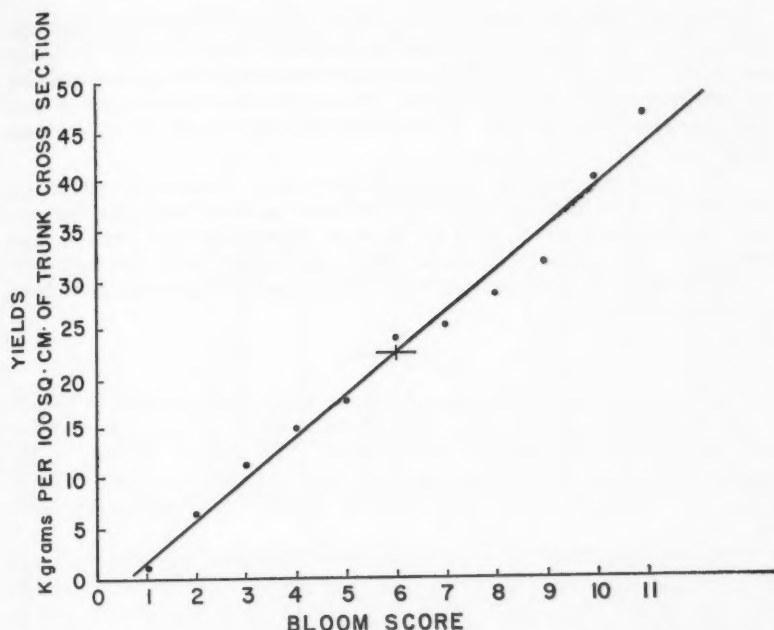


FIGURE 1. The relationship between the amount of bloom and yield of apples.

The cross-sectional area of each tree was measured in the fall at a point marked by a nail about 12 inches above ground level. The trunk cross-section recorded in the fall was related to the yield of that year. The amount of bloom per tree for the years 1949 to 1958, inclusive, was recorded visually by the writer on a numerical scale of 1 to 11. Class 1 indicates no or very scanty bloom; 11 indicates maximum bloom. This empirical method of recording bloom was the only one physically possible in the limited time each spring, and thus there may have been slight inconsistencies from year to year, from day to day, or even within one day. However, it is felt that limitation of this measurement to one observer reduced the degree of error. Yields for the 10 years of the test are presented in kilograms per 100 square centimetres of trunk cross-section.

Fundamentally, the study comprised the triple records of 12,313 trees—their trunk cross-sectional areas, bloom and yield. This average of 1,231 trees per year, ranging from the high of 1,936 in 1950 to the low of 602 in 1955, rules out any selection for uniformity, but the trees were grouped and averaged on the basis of trunk cross-section for each year and for the 10-year total.

Data were available from trees where fruit sizes were determined for other purposes. These included records for 4 years for Golden Russetts and 3 years for McIntosh from a cultural project. The 6 years' records for Rome Beauty were from a fertilizer test.



Two methods of sampling were used. One to four fire crates, depending on the yield, were taken at random, weighed and the apples counted. The second method was to take a sample of 100 apples, consisting of a uniform number of apples from a certain location from each of at least half the crates from a tree. If the yield was very small, all apples were included.

The available weather records limited the evaluation of pollinating conditions. The number of hours of sunshine on days when the temperature reached both 60° F. and 65° F. were determined and used as such measures. The period from first McIntosh bloom until the mid-point between full bloom and when all McIntosh petals had fallen was considered the bloom period.

### RESULTS

The average trunk cross-sectional area of the larger group of trees in 1949 was considerably less than that of the much smaller group in 1958—347 compared to 503 square centimetres. The growth increase in the trunk, averaging 8.97 per cent per year, but not shown in the presented data, more than balanced the removals which were mostly the older, larger trees.

Before examining the relationship between bloom and yield per unit area of trunk cross-section, the uniformity of the yields of the bloom classes was examined. Since eleven bloom classes for 10 years totalled 110, to examine them all would have involved a very large amount of computation. Therefore, ten groups were chosen at random from those containing 50 to 90 trees.

The coefficients of variability of these groups were determined and found to average 54.79 with a range from 38.0 to 85.1. This is comparable with the coefficients of variability of apple tree yields as reported by other workers (4, 5, 6).

TABLE 1.—THE MEAN YIELD OF APPLE TREES PER UNIT OF TRUNK CROSS-SECTION IN RELATION TO THE AMOUNT OF BLOOM

Bloom score <sup>2</sup>	Year										Mean <sup>1</sup>
	1949	1950	1951	1952	1953	1954	1955	1956	1957	1958	
	Kilograms/100 cm <sup>2</sup> trunk c/s										
1	0.27	2.30	0.88	0.47	0.45	1.85	0.51	0.82	2.28	4.43	0.89
2	1.35	9.20	5.36	4.45	3.93	10.08	4.89	7.15	11.93	8.52	6.05
3	4.38	13.55	7.67	10.60	6.73	17.31	10.55	14.44	22.61	14.16	11.36
4	5.51	17.89	9.82	13.03	9.77	22.37	15.71	22.28	26.20	21.89	15.01
5	11.62	19.31	14.83	13.73	12.29	21.40	20.59	26.63	28.82	29.10	18.04
6	14.35	23.93	19.14	20.30	20.38	28.63	25.44	38.66	33.06	31.28	23.13
7	16.32	21.29	22.04	21.35	23.12	31.63	27.96	34.36	35.16	30.74	23.88
8	20.27	19.63	24.15	27.67	23.93	36.95	29.66	41.42	43.59	35.41	29.10
9	27.22	23.39	24.82	28.47	24.79	39.69	35.04	48.70	48.33	44.58	31.77
10	34.86	29.57	31.57	37.75	32.23	47.61	41.58	53.20	57.57	47.85	39.06
11	28.84	35.65	46.38	42.13	41.35	50.67	53.75	52.75	55.17	62.82	46.48
Mean <sup>1</sup>	15.77	26.61	24.00	22.12	26.46	32.26	45.02	31.42	49.02	45.28	29.61

<sup>1</sup> Weighted mean

<sup>2</sup> Score "1"—trees with no bloom or very few blooms; score "11"—maximum bloom



TABLE 2.—THE MEAN<sup>1</sup> YIELD OF CORTLAND, MCINTOSH, NORTHERN SPY, AND ALL VARIETIES PER UNIT OF TRUNK CROSS-SECTION IN RELATION TO THE AMOUNT OF BLOOM, 1949-1958

Bloom score <sup>2</sup>	Cortland	McIntosh	Northern Spy	All varieties
Kilograms/100 cm <sup>2</sup> , trunk c/s				
1	1.54	0.88	0.95	0.89
2	7.11	5.99	6.25	6.05
3	11.75	13.11	11.57	11.36
4	17.10	16.06	19.87	15.01
5	22.77	18.82	20.55	18.04
6	28.30	22.95	27.71	23.13
7	37.20	30.66	27.35	23.88
8	37.47	33.97	36.51	29.10
9	45.63	38.78	40.05	31.77
10	50.98	45.00	51.08	39.06
11	55.38	55.07	57.74	46.48
Mean	47.81	39.96	32.80	29.61

<sup>1,2</sup>See footnotes, Table 1

The weighted average yield of all trees in Class 11 for the 10 years of the study was 46.48 kilograms per 100 square centimetres of trunk cross-section as shown in Table 1, and there was a steady decrease in yield with decreasing amount of bloom. A comparison of the three major varieties, Cortland, McIntosh and Northern Spy, with each other and with "all varieties" (Table 2) showed that these three varieties produced more heavily than the "all-variety" average. However, the yields of each indicated a linear relationship between bloom and yield. The Cortland trees were the heaviest producers per unit (100 sq. cm.) of trunk cross-section, and the weighted average yields for all classes showed Cortland, McIntosh, Northern Spy and "all varieties" in decreasing order of yield.

The average yields of the different bloom classes of all varieties and also for the varieties Cortland, McIntosh and Northern Spy gave almost straight line relationships, the correlation coefficients and regression equations being as follows:

All varieties	$r = +.9911$	$y = 4.14 x - 2.58$
Cortland	$r = +.9976$	$y = 5.47 x - 4.15$
McIntosh	$r = +.9923$	$y = 5.02 x - 4.52$
Northern Spy	$r = +.9885$	$y = 5.35 x - 4.88$

where:  $r$  = correlation coefficient $y$  = kilograms of fruit/100 cm<sup>2</sup> trunk C/S $x$  = bloom score, 1 none, 11 maximum.

The analysis of variance (Tables 3 and 4) computed from the data in Table 1 showed a highly significant effect of bloom class on yield. The linear component of the sum of squares for yield was obtained from the table of orthogonal polynomials (2). It is highly significant (Table 4), and indeed accounts for 98 per cent of the yield sum of squares.

With Golden Russett and McIntosh the average size of the fruit from the trees with the different scores was approximately equal. In the

TABLE 3.—ANALYSIS OF VARIANCE OF MEAN YIELDS IN KILOGRAMS PER 100 SQUARE CENTIMETRES OF TRUNK CROSS-SECTION

Source of variation	D.F.	Mean square	F
Years	9	443.7669	27.7743
Bloom classes	10	2008.9249	125.7338
Error	90	15.9776	
Total	109		

TABLE 4.—ANALYSIS OF VARIANCE OF YIELDS IN KILOGRAMS PER 100 SQUARE CENTIMETRES OF TRUNK CROSS-SECTION FROM REGRESSION OF BLOOM SCORES

Source of variation	D.F.	Mean square	F
Linear	1	19869.8559	815.1067
Residual	9	24.3770	
Total	10		

TABLE 5.—THE AVERAGE FRUIT WEIGHT IN KILOGRAMS PER 100 APPLES OF GOLDEN RUSSETT, MCINTOSH AND RED ROME BEAUTY FROM TREES GROUPED ACCORDING TO BLOOM SCORE

Bloom score	Variety		
	Golden Russett	McIntosh	Rome Beauty
1	9.10	10.80	—
2	8.91	—	10.85
3	8.43	10.16	10.97
4	8.26	9.30	9.50
5	9.19	10.27	7.79
6	8.81	11.85	9.40
7	9.12	10.93	7.03
8	9.39	10.76	8.72
9	9.65	10.55	9.04
10	9.43	10.16	9.05
11	9.65	10.65	8.58

instance of Rome Beauty the fruit produced on trees with bloom scores of 2 and 3 were larger than from the trees in the higher bloom score classes. The difference for the trees with bloom scores of from 4 to 11 was probably not significant. These data are shown in Table 5.

The weather conditions during the bloom period were studied to ascertain if there was a relationship between such conditions and the yield of trees in the "1 bloom" score classes, where comparatively heavy yields

were obtained in some years. The coefficients of correlation between the hours of sunshine during the bloom period during days when the maximum temperatures were above both 60° F. and 65° F. and yields were insignificant, being respectively 0.43 and 0.54 with 8 degrees of freedom.

### DISCUSSION

Under the conditions on the Experimental Farm at Kentville, Nova Scotia, in the 10 years 1949 to 1958, the yield of apple trees per unit area of trunk cross-section was directly proportional on unthinned trees to the amount of bloom. It would appear that the increased uniformity of single varieties about balanced the smoothing effect of the large numbers when all varieties were studied together. This is shown by the coefficient of correlation.

The data clearly show that, under the conditions of the orchard studied, yields increased as the amount of bloom increased and it is concluded that an "11 score" bloom was required on the average to produce a full crop.

### ACKNOWLEDGEMENTS

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# FACTORS INFLUENCING THE COLOUR OF POTATO CHIPS<sup>1</sup>

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## ABSTRACT

Experiments using model systems and potato juices treated with ion exchange resins showed that the fundamental browning which occurred during chipping was due to the reaction between amino acids and reducing sugars. Browning also occurred in the presence of amino acids and hydrolyzed sucrose. Evidence is presented to show that conditions favouring hydrolysis do prevail during chipping but the extent to which it occurs, and the consequent browning, are dependent on the degree of acidity present during frying.

Studies revealed that the initial presence of reducing sugars was not a requirement for the browning reaction, provided sucrose was present and conditions were favourable for its hydrolysis.

Inorganic constituents, *per se*, were found to have little colour-enhancing effect. However, their importance, particularly that of phosphate, would seem to be in the promotion of sucrose hydrolysis.

Colour has long been recognized as the most important attribute of potato chips, and various investigators (3, 4, 5, 6, 9, 11) have shown a high content of reducing sugars in tubers to be associated with dark-coloured chips. More recently a reaction between amino acids and reducing sugars has been demonstrated to be at least partially responsible for the browning of chips (7, 8, 10).

The fact that tubers with a low content of reducing sugars do not always produce chips of desirable colour suggests that other factors may be involved. The study reported here was an attempt to determine these factors and their effect on chip colour.

## MATERIALS AND METHODS

Two-pound samples of each of the following varieties of potatoes, Irish Cobbler, Warba and Kennebec, were used. Samples of the first two varieties were procured from a local chip plant and produced dark unattractive chips. The Kennebec variety was grown at the Research Station, Kentville, N. S., and produced light-coloured attractive chips.

The potatoes were peeled, diced and ground in an "Osterizer" blender. The juice was pressed from the ground material and centrifuged to remove starch. To ascertain if all the browning reactants were contained in the juice, a portion of the pressed cake was fried in fat. Only a faint brown colour was evident. When the cake was washed once with distilled water before frying, the colour was negligible. Heat-coagulable protein, which was found to play an inconsequential part in the browning reaction, was next removed by heating the juice to 80° C. and filtering. Following filtration, conductivity was measured with a Solu-Bridge Model RD-15 soil tester, amino nitrogen was determined by the method of Yemn and Cocking (14), and reducing sugars and sucrose by the method of Munson and Walker (2).

<sup>1</sup> Contribution No. 1010 from Research Station, Canada Department of Agriculture, Kentville, N. S.

Paper discs, 10 cm. in diameter, were cut from Whatman No. 2 chromatograph paper, saturated with juice and fried in fat (Primex) for  $1\frac{1}{2}$  minutes at  $375^{\circ}$  F. in a thermostatically controlled Moffat deep fat frier.

Portions of juice from tubers of the Irish Cobbler and Warba varieties were passed through a column of strongly acidic IR-120 cation exchange resin\*, and further purified by passage through a column of weakly basic IR-4B anion exchange resin\*. Other portions of juice of these two varieties were passed through a mixed-bed system, comprised of two parts by volume of weakly acidic IRC-50 resin\* and one part of weakly basic IR-45 resin\*. Conductivity, amino nitrogen, reducing sugars, and sucrose were determined on the four effluents, and paper discs saturated with these effluents were fried as previously described. Reflectance readings were made on all fried discs, using a Photovolt Reflection Meter. A blue filter was employed since it provided a greater range than green or amber. Two discs were read in each instance and five readings were made on each disc.

In order to determine the soluble acidic and basic constituents removed from the juice by ion exchange resins, the IR-120 column was washed with distilled water and treated with a 5 per cent solution of hydrochloric acid. The IR-4B column was similarly washed and treated with a 5 per cent solution of sodium hydroxide. With some slight modification, the Winton and Winton method (13) was followed in analysing the eluates for potassium, sodium, calcium, magnesium, phosphorus, sulphur and chlorine. The same constituents were also determined in the ash of untreated juice.

To obtain an estimate of chip colour attributable to amino acids, sugars, phosphorylated sugars, and soluble cations and anions, solutions were made up containing these substances in various combinations. Paper discs were saturated with the solutions and fried as previously described.

The amino acid solutions contained: alanine, arginine, aspartic acid, asparagine, glutamic acid, leucine, lysine, phenylalanine, serine, threonine, tyrosine and valine. The quantities added were governed by the amino nitrogen content found for tubers of the Irish Cobbler, Warba and Kennebec varieties and the proportion of each amino acid to the total amino nitrogen content was the same as reported by Thompson and Steward (12).

The amounts of dextrose and sucrose employed were identical with the amounts of reducing sugars and sucrose found in the juice of tubers of the three varieties. Two phosphorylated sugars, glucose-6-phosphate and fructose-6-phosphate, both in free form, were utilized. The amounts used were based on the amounts of phosphorus removed by the IR-4B column from juice of the variety Irish Cobbler.

The soluble cations and anions used were contained in mono-hydrogen potassium phosphate ( $K_2HPO_4$ ), di-hydrogen potassium phosphate ( $KH_2PO_4$ ) and potassium chloride (KCl). The quantities added were governed by the amounts of potassium, chlorine, and phosphorus found in the juice of Irish Cobbler tubers.

In order to determine the quantity of sucrose hydrolyzed by various salts, amino acids and phosphorylated sugars, solutions containing these

\*Manufactured by Resinous Products Division, Rohm & Haas Co., Philadelphia, Pa.

TABLE 1.—CHEMICAL COMPOSITION OF JUICE FROM KENNEBEC, IRISH COBBLER AND WARBA POTATOES AND EFFECT OF ION EXCHANGE RESINS ON CONSTITUENTS PRESENT IN JUICE OF THE IRISH COBBLER AND WARBA VARIETIES

Constituents	Kennebec	Irish Cobbler			Warba		
	Untreated	Untreated	Passed through IRC-50 + 1R-45 resins	Passed through 1R-120 and 1R-4B resins	Untreated	Passed through IRC-50 + 1R-45 resins	Passed through 1R-120 and 1R-4B resins
Reducing sugars (mg./ml.)	Nil	1.50	1.50	1.30	4.30	4.25	2.80
Sucrose (mg./ml.)	2.00	4.70	4.70	3.00	4.03	4.00	2.25
Amino nitrogen (mg./100 ml.)	68.2	116	90	Nil	107	107	0.71
Conductivity (mhos $\times 10^{-4}$ )	1,000	1,000	350	10	1,000	200	10
Reflectance values of paper discs	28.0	17.9	20.2	50.0	13.5	19.0	55.0

substances in various combinations were heated at 70° C. for different periods in a water bath and the amount of reduced copper determined by the method of Munson and Walker (2).

## RESULTS

The chemical composition of juice from Kennebec, Irish Cobbler and Warba potatoes, and the effect of treatment with ion exchange resins on constituents present in juice of the Irish Cobbler and Warba varieties, are shown in Table 1.

The greatest differences in the three untreated juices were in content of reducing sugars which were absent in the Kennebec sample and highest in Warba. The Kennebec sample also contained less sucrose and amino nitrogen than either of the other two varieties. Paper discs saturated with untreated juice of the Kennebec sample and fried had a photovolt reflectance reading of 28 and a light brown colour. Discs saturated with untreated juice of the Irish Cobbler and Warba samples and fried gave reflectance readings of 17.9 and 13.5 respectively. These discs had a dark brown colour.

Treatment of juice of Irish Cobbler and Warba samples with ion exchange resins IRC-50 and IR-45 resulted in practically no change in sugar content, only a slight reduction in amino nitrogen but a considerable decrease in conductivity. Discs saturated with the effluents and fried were an undesirable brown colour. Treatment of juices of the same varieties with ion exchange resins IR-120 and IR-4B resulted in a decrease in sugar content, with sucrose being removed to a slightly greater extent than reducing sugars, and a virtually complete removal of amino acids and ionizable material as measured by conductivity. Discs treated with the effluents and fried were practically devoid of any brown colour.

The results of analyses of the eluates from ion exchange resins IR-120 and IR-4B following passage of juice of Irish Cobbler tubers and results of analysis of the ash of untreated juice of the same variety are shown in Table 2. Except for phosphorus and sulphur there was little difference



between the eluates and ash with respect to the amounts of the various constituents found. Potassium was present in greater quantity than any other ion.

Photovolt Reflection Meter readings were made on paper discs which, before being fried, had been saturated with solutions containing amino acids, sugars, salts and phosphorylated sugars in various combinations. Results are presented in Table 3. Either dextrose or amino acids alone

TABLE 2.—AMOUNTS OF CONSTITUENTS REMOVED FROM JUICE OF IRISH COBBLER TUBERS BY ION EXCHANGE RESINS IR-120 AND IR-4B AND THE CHEMICAL COMPOSITION OF THE ASH OF THE UNTREATED JUICE

Constituent	Eluates mg./ml.	Untreated juice (ashed) mg./ml.
Potassium (K)	3.00	3.47
Sodium (Na)	0.10	0.19
Calcium (Ca)	0.06	0.07
Magnesium (Mg)	0.05	0.05
Phosphorus (P)	0.23	0.79
Sulphur (S)	Trace	0.27
Chlorine (Cl)	0.68	0.79

TABLE 3.—REFLECTANCE VALUES OF PAPER DISCS TREATED WITH VARIOUS COMBINATIONS OF AMINO ACIDS, SUGARS, SALTS, AND PHOSPHORYLATED SUGARS AND FRIED IN FAT AT 375° F. FOR 1½ MINUTES

Reactants	pH of reactants	Per cent reflectance dextrose levels (mg./ml.)		
		0	1.6	4.3
No additions	7.0	64.0	62.9	61.9
Amino acids <sup>1</sup>	3.9	53.7	40.5	25.9
Amino acids <sup>1</sup> + sucrose <sup>2</sup>	3.9	37.9	29.9	21.5
Amino acids <sup>1</sup> + salts <sup>3</sup>	7.5	56.0	37.0	23.5
Amino acids <sup>1</sup> + salts <sup>4</sup>	4.1	48.0	34.6	22.7
Amino acids <sup>1</sup> + salts <sup>5</sup>	4.0	53.2	37.4	25.5
Amino acids <sup>1</sup> + salts <sup>6</sup>	5.9	53.3	36.9	25.3
Amino acids <sup>1</sup> + glucose-6-phosphate <sup>7</sup>	3.2	45.0	33.7	23.4
Amino acids <sup>1</sup> + fructose-6-phosphate <sup>7</sup>	3.0	47.6	35.1	23.1
Amino acids <sup>1</sup> + sucrose <sup>2</sup> + salts <sup>3</sup>	7.5	51.8	36.3	20.8
Amino acids <sup>1</sup> + sucrose <sup>2</sup> + salts <sup>4</sup>	4.1	24.6	19.9	16.1
Amino acids <sup>1</sup> + sucrose <sup>2</sup> + salts <sup>5</sup>	4.0	29.6	24.5	17.0
Amino acids <sup>1</sup> + sucrose <sup>2</sup> + salts <sup>6</sup>	5.9	29.4	24.4	17.3
Amino acids <sup>1</sup> + sucrose <sup>2</sup> + glucose-6-phosphate <sup>7</sup>	3.2	25.8	20.6	14.5
Amino acids <sup>1</sup> + sucrose <sup>2</sup> + fructose-6-phosphate <sup>7</sup>	3.0	23.3	19.4	15.5
Amino acids <sup>1</sup> + sucrose <sup>2</sup> + salts <sup>3</sup>	6.0	41.0	—	—

<sup>1</sup>100 mg. of amino N/100 ml.

<sup>2</sup>4.7 mg./ml.

<sup>3</sup>6.3 mg. K<sub>2</sub>HPO<sub>4</sub> + 1.5 mg. KCL/ml.

<sup>4</sup>6.0 mg. KH<sub>2</sub>PO<sub>4</sub> + 1.5 mg. KCL/ml.

<sup>5</sup>1.0 mg. KH<sub>2</sub>PO<sub>4</sub> + 1.5 mg. KCL/ml.

<sup>6</sup>1 mg. K<sub>2</sub>HPO<sub>4</sub> + 2 mg. KH<sub>2</sub>PO<sub>4</sub> + 1.5 mg. KCL/ml.

<sup>7</sup>2.1 mg./ml.

<sup>8</sup>68 mg. of amino N/100 ml.

<sup>9</sup>2.0 mg./ml.



TABLE 4.—THE EFFECT, AFTER HEATING FOR VARIOUS PERIODS AND AT VARIOUS pH LEVELS IN A WATER BATH AT 70° C., OF VARIOUS COMBINATIONS OF AMINO ACIDS, SALTS AND PHOSPHORYLATED SUGARS ON HYDROLYSIS OF SUCROSE

Reactants	pH	Per cent sugar (as sucrose) hydrolyzed with heating		
		½ hour*	2 hours*	8 hours**
Amino acids <sup>1</sup> + sucrose <sup>2</sup>	3.9	Nil	17.7	—
Amino acids <sup>1</sup> + sucrose <sup>2</sup> + salts <sup>3</sup>	7.3	Nil	Nil	Nil
Amino acids <sup>1</sup> + sucrose <sup>2</sup> + salts <sup>4</sup>	4.1	Nil	20.2	—
Amino acids <sup>1</sup> + sucrose <sup>2</sup> + salts <sup>5</sup>	4.0	Nil	24.0	—
Amino acids <sup>1</sup> + sucrose <sup>2</sup> + salts <sup>6</sup>	5.9	Nil	Nil	2.8
Glucose-6-phosphate <sup>7</sup>	2.3	18.2	—	—
Sucrose <sup>2</sup> + glucose-6-phosphate <sup>7</sup>	2.3	116.5	—	—
Amino acids <sup>1</sup> + sucrose <sup>2</sup> + glucose-6-phosphate <sup>7</sup>	3.2	62.4	—	—
Fructose-6-phosphate <sup>7</sup>	2.2	4.1	—	—
Sucrose <sup>2</sup> + fructose-6-phosphate <sup>7</sup>	2.2	103.8	—	—
Amino acids <sup>1</sup> + sucrose <sup>2</sup> + fructose-6-phosphate <sup>7</sup>	3.0	60.6	—	—

\*Amount of solution heated — 8 ml.

\*\*8 ml. of solution concentrated to 1 ml. before heating

<sup>1</sup>100 mg. of amino N/100 ml.<sup>2</sup>4.7 mg./ml.<sup>3</sup>6.3 mg. K<sub>2</sub>HPO<sub>4</sub> + 1.5 mg. KCL/ml.<sup>4</sup>6.0 mg. KH<sub>2</sub>PO<sub>4</sub> + 1.5 mg. KCL/ml.<sup>5</sup>1.0 mg. KH<sub>2</sub>PO<sub>4</sub> + 1.5 mg. KCL/ml.<sup>6</sup>1.0 mg. K<sub>2</sub>HPO<sub>4</sub> + 2 mg. KH<sub>2</sub>PO<sub>4</sub> + 1.5 mg. KCL/ml.<sup>7</sup>2.1 mg./ml.

produced virtually no brown colour. Similar findings were obtained for amino acids and salts or amino acids and phosphorylated sugars. At the highest level dextrose was an effective colour producer in combination with amino acids. Even at the intermediate level of dextrose some colour was formed with amino acids. On the contrary, differences in pH and the addition of salts or phosphorylated sugars did not noticeably increase the colour, irrespective of dextrose level.

Sucrose in combination with amino acids caused some browning which was intensified in the presence of dextrose, particularly at the highest level. At pH 7.5 very little brown colour resulted from the reaction between sucrose, amino acids and salts. In fact the amount of colour was considerably less than that produced at pH 3.9 by amino acids and sucrose alone. At all other pH values the brown colour was increased when salts or phosphorylated sugars were added to amino acids and sucrose or amino acids, sucrose and dextrose.

The amounts of sucrose hydrolyzed in the presence of different combinations of amino acids, salts and phosphorylated sugars are shown in Table 4. After 2 hours' heating, some inversion of sucrose by amino acids alone or by amino acids and salts occurred when the pH was in the range 3.9 to 4.1. At pH 5.9 a limited amount of inversion took place in the presence of amino acids and salts after concentration and 8 hours' heating, whereas at pH 7.3 no inversion occurred, irrespective of concentration or time of heating. Phosphorylated sugars alone inverted all the sucrose in one-half hour. The addition of amino acids reduced acidity and less sucrose was hydrolyzed.

## DISCUSSION

The results herein reported substantiate the findings of others (7, 8, 10) that the fundamental browning reaction is between amino acids and reducing sugars. The reaction is not substantially affected by the presence of acidic or basic constituents. Considerable browning can occur in the presence of sucrose and amino acids providing conditions are favourable for sucrose hydrolysis. The findings strongly suggest such conditions do exist during chipping and this view is supported by the following facts:

- (a) At pH 7.5, very little browning occurred with sucrose, amino acids and salts and the depth of the brown colour was considerably less than that produced at pH 3.9 by sucrose and amino acids alone (Table 3). At all other pH values studied, including that of potato juice (5.8 – 6.0), considerable brown colour was formed with sucrose, amino acids and salts or with sucrose, amino acids and phosphorylated sugars.
- (b) at pH 7.3 no sucrose was hydrolyzed by heating or concentration (Table 4), but at all other pH values including that of potato juice, some sucrose was hydrolyzed when salts, amino acids or phosphorylated sugars were present either alone or in combination.
- (c) Experimental studies by the writers\* revealed that the pH of some tuber juice decreased from 6.0 to 4.9 following an eightfold concentration at 100° C. This acidity, in conjunction with thermal conditions, makes it reasonable to expect hydrolysis of sucrose during chipping, although Habib and Brown (8) could find no evidence of this.

\*Unpublished data.

In the present investigation amino acids were indispensable for the browning reaction. Results in Tables 1 and 3 suggest that browning could conceivably occur in the absence of sucrose, inorganic constituents or phosphorus compounds but not in the absence of amino acids. The fact that browning occurred in discs treated with juice of Kennebec tubers also showed that the initial presence of reducing sugars in the raw juice was not always necessary for browning.

There was no evidence that the phosphate ion, or any other ion, *per se*, was responsible for significant amounts of the colour formed during the browning reaction. However, some inorganic constituents—particularly phosphate since it is the chief acid-forming inorganic element (13)—would be important in the browning reaction by providing conditions favourable to sucrose hydrolysis. Results (Table 4) corroborate this since they indicate that it is the acidity of the medium rather than the constituents themselves that is important in hydrolysis.

It is conceivable, too, that a large portion of the phosphorus may come from phosphorylated sugars since the phosphate moiety of the esters is a strong acid and loosely held. Moreover, Arreguin and Bonner (1) report considerable quantities of glucose-6-phosphate and fructose-6-phosphate in tubers, and the presence of the orthophosphate ion in tuber juice has been demonstrated by the writers.

Treatment of Irish Cobbler and Warba juice with ion exchange resins indicated that salts were not responsible for much of the browning in chips. The fact remains, however, that paper discs dipped in these juices prior to and following treatment with the resins IRC-50 and IR-45 and fried, showed some differences in colour. The differences may reflect incomplete

hydrolysis of sucrose because of the removal of ions by the resins. Results (Table 3) showed that salts were more effective in increasing colour with sucrose and amino acids than with dextrose and amino acids. There is the possibility, too, that differences in pH, following ion exchange treatment of juices, may be responsible for some of the disparity in colour. However, no pH measurements were made on ion exchange treated potato juice.

The colour produced by hydrolyzed sucrose and amino acids would probably be masked in chips from tubers with an initially high content of reducing sugars but would become increasingly important in chips having a relatively low initial content. Any change in pH of tubers during chipping would also be important since hydrolysis of sucrose, hence browning, increases with increasing acidity.

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# THE INFLUENCE OF VARIETY, TEMPERATURE, AND STAGE OF GROWTH ON THE RESPONSE OF SPRING BARLEY TO PHOTOPERIOD<sup>1</sup>

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## ABSTRACT

Olli and Vantage barley were grown in plant growth chambers under 27 combinations of photoperiod at continuous temperatures of 55° and 75°F. in two consecutive experiments. The photoperiod treatments (8, 16 and 24 hours) were applied during seedling to internode elongation (Stage I), internode elongation to heading (Stage II), and heading to maturity (Stage III) of the first culm.

Increases in photoperiod during Stage I reduced the number of days from seedling to internode elongation and heading but increased the number of days from heading to maturity, reduced leaf number and height, weight of stem and head, stem length, head length, number of florets and kernels per head, and fertility for the first culm. Time of initiation of tillering relative to elongation of the first internode of the first culm was advanced for Olli but delayed for Vantage with the increases in photoperiod. Tillering extended over a longer period and tillers were more numerous under 16- than under 8- or 24-hour photoperiods during Stage I.

Increases in photoperiod during Stage II reduced the number of days from internode elongation to heading but generally increased leaf, stem and head development, and fertility for the first culm, and increased the number of fertile tillers. Increases in photoperiod during Stage III reduced the number of days from heading to maturity of the first culm and increased the number of fertile tillers.

An increase in the temperature from 55° to 75°F. reduced the duration and extent of development of the first culm, delayed commencement and reduced duration of tiller initiation, and reduced the number and extent of development of the tillers. The influence of temperature decreased with increase in length of photoperiod.

Olli and Vantage differed in duration of leaf initiation and number of leaves associated with the first culm, in kernel weight and in the relative time of initiation of tillering. They were essentially similar in duration and extent of stem and head development and fertility for the first culm, in the duration of tillering and number of tillers produced, and in the extent of stem, head and kernel development of the tillers.

There were significant associations of number of days from seedling to internode elongation with number of leaves on the first culm, among stem length, head length, number of florets per head and weight of the stem and head, and of number of tillers with duration of tillering.

## INTRODUCTION

Barley and other spring cereals could be grown on much of the potentially arable land of Northwestern Canada<sup>2</sup>. However, the development of well adapted, early maturing varieties is prerequisite to a full realization of the potential of these areas. Breeding for this adaptation is hindered by the lack of information on varietal response to photoperiod and temperature.

<sup>1</sup>Based on a thesis submitted to the School of Graduate Studies, University of Nebraska, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

<sup>2</sup>Report of Uniform Alcan Cereal Tests, Reports No. 1-6 (mimeographed). Experimental Farm, Beaverlodge, Alberta, and Experiment Station, Palmer, Alaska, 1952-57.

Investigations with wheat and oats indicate that the response to photoperiod may be conditioned by variety, temperature and stage of plant development. No detailed studies have been conducted with barley and all but the most recent studies with wheat and oats have been conducted in the field or greenhouse where control of the environment is inadequate.

It has been reported that long photoperiods approaching 24 hours as compared with intermediate photoperiods approximating 16 hours reduce leaf number (1, 9) and total leaf weight of wheat (1). There is a general acceleration of time of heading with increase in photoperiod for wheat (2, 6, 10) and for oats (14). For wheat, more rapid stem elongation under long as compared with intermediate photoperiods (1, 4) contributes to earlier heading. The reduction of final stem length associated with the increased rate of elongation indicates a restriction in the duration of stem elongation. With wheat there are reductions in the length of head, number of spikelets per head, number of kernels per head and kernel weight (12), and fertility (11, 12) under both long and short photoperiods as compared with intermediate photoperiods. Long photoperiods reduce the duration of tillering of wheat (1, 2, 3, 5) and the number of head bearing tillers of wheat (12) and of oats (13, 15). However, photoperiod does not always influence the number of potential tiller buds of oats (13).

The lack of precise information on the response of barley to photoperiod dictated the scope of the present study. The primary objective was to obtain fundamental information on the influence of photoperiod on the rate, duration and extent of development of the vegetative and reproductive parts during three well defined stages of growth. Of equal importance was a study of the extent to which the response to photoperiod during a given stage of development was conditioned by variety, prior photoperiod and temperature. Knowledge of the developmental relationships of the leaves, stem and head of the main axis, and of the tillers was considered essential to an understanding of the photoperiod and temperature responses.

#### MATERIALS AND METHODS

Olli, an early, and Vantage, a late maturing variety of barley were each grown under all combinations of 8-, 16- and 24-hour photoperiods during three stages of growth of the first culm, namely: seedling to internode elongation (Stage I); internode elongation to heading (Stage II); and heading to maturity (Stage III). Thus, there were three photoperiod treatments during Stage I, nine combinations of present and prior photoperiod during Stage II and 27 combinations during Stage III. The first phase of the experiment was conducted at a continuous temperature of 55°F. and a second phase at 75°F.

The experiments were conducted in six 9 × 2.5 × 8 foot plant-growth chambers. Each chamber was illuminated by a 2 × 8 foot bank of fluorescent tubes placed on 2-inch centres. The tubes maintained a light intensity of from 1300 to 1500 f.c. at 2 feet from source as measured by a Weston Model 756 Illumination Meter. The seed was germinated with the surface of the growth medium 18 inches from the light source and the tops of the plants kept within 12 to 18 inches by lowering the containers

by 6-inch intervals. Sufficient air of the desired temperature was forced continuously, by a 1200 C.F.M. air-conditioning unit, through each of the chambers to maintain the temperature within  $\pm 1^\circ\text{F}$ . of the temperature of the air at source.

Plants were grown in  $12 \times 6 \times 12$  inch plywood containers. The containers were filled with water-saturated exploded mica (Vermiculite) compressed to approximately one-half the dry volume. Ten plants spaced 2 inches apart were grown to maturity in each container. The vermiculite was saturated with nutrient solution 1 week after seeding and at 10-day intervals thereafter. The nutrient solution used was Hoagland and Arnon's solution No. 2 (8) modified by the substitution of versene iron chelate for the iron salt.

Duplicate treatments were grown in separate chambers. Treatments were moved from one photoperiodic chamber to another when the first culms of eight of the ten plants reached the designated stage of development. Dates of initiation of elongation of the first internode, heading and maturity or termination of development were recorded for the first culm of each plant. The first internode was considered to have commenced elongation when the first node could be distinguished above the base of the culm. The location of the large nodes of barley can be detected readily by sliding the fingers lightly up and down the culm. The number and height of the leaves (measured from ground level to the tip of the tallest leaf) associated with the development of the first culm were recorded for each plant at 4-day intervals commencing 7 days after seeding.

TABLE 1.—EXAMPLE OF ANALYSIS OF VARIANCE APPLIED TO COMPLETE DATA

Source of variation	Degrees of freedom	Mean squares	
		Number of leaves	Weight of stem and head in grams
Temperature	1	3.28**	3.112**
Replicate in temperature	2	0.37	0.003
Photoperiod Stage I	2	636.64**	1.780**
Photoperiod Stage II	2	2.38**	2.120**
PI $\times$ P II	4	0.47	0.108**
PI $\times$ T	2	7.66**	1.524**
P II $\times$ T	2	0.50	0.397**
PI $\times$ P II $\times$ T	4	0.17	0.086**
Error (a)	16	0.19	0.008
Variety	1	1,027.92**	0.006
V $\times$ T	1	1.18**	0.038
V $\times$ P I	2	258.30**	0.009
V $\times$ P II	2	1.28**	0.012
V $\times$ T $\times$ P I	2	1.56**	0.046*
V $\times$ T $\times$ P II	2	0.56*	0.020
V $\times$ P I $\times$ P II	4	0.64**	0.050**
Error (b)	22	0.13	0.009
Individual treatment	144	0.07	0.004

NOTE: Single or double asterisks indicate that the F-values exceed the 5 or 1 per cent levels of probability, respectively.



At the termination of development of the first culm, the plants were lifted from their containers and dissected. Length of stem, length of head, and number of florets and kernels per head were recorded for the first culm and for each tiller. Number of fertile, sterile, immature and rudimentary tillers was also recorded for each plant. Leaves and leaf sheaths, stems and heads of the first culm, and stems and heads of the tillers were dried at 90°C. for 5 hours and weighed separately for each treatment. Kernels were air-dried and weighed.

An example of the analysis of variance for response to temperature, variety, and photoperiod during Stages I and II is presented in Table 1. The mean squares for number of leaves, and weight of stem and head are included to indicate the precision obtained with the controlled environmental conditions.

The analyses were complicated by certain unavoidable irregularities in design. As effects of temperature were determined in two consecutive experiments, the temperature x replication interaction could not be extracted as the error variance for measuring response to temperature. For any response determined during Stage I, photoperiod constituted the first and variety the second split within each temperature. However, change of photoperiod during successive stages of growth was accomplished by transferring the treatments to different chambers, thus eliminating the influence of uncontrolled variation among chambers. For these reasons, the responses to temperature and photoperiod during one or more stages of growth were tested against a common error variance.

Olli and Vantage were not always together because of the varietal difference in rate of growth but were moved through the same chambers. Thus, despite a time factor, they were considered to be paired and the variances attributed to variety and to the interactions of variety with photoperiod and temperature were tested against a second error variance. The final apparent irregularity was caused by basing all analyses on the full complement of 216 entries. Thus, in analysing responses that terminated during Stage I, there were nine entries for each treatment and during Stage II three entries. These were analysed as individuals.

Where data were incomplete because of failure of the plants to develop to the required stage under certain treatments, differences in average response were measured by "t" tests based on paired data (i.e., for comparisons of response to temperature the data were paired on the basis of variety and photoperiod). Relationships were measured by coefficients of correlation and linear regression.

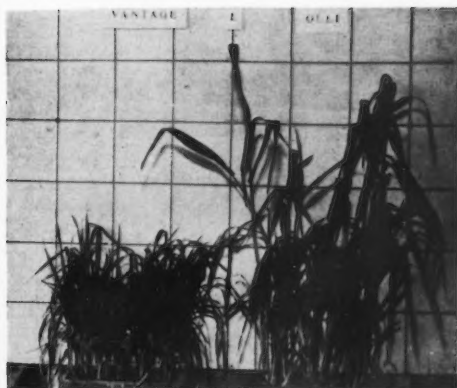
## RESULTS AND DISCUSSION

### PHENOLOGY AND MORPHOLOGY OF THE FIRST CULM

#### *Elongation of the First Internode*

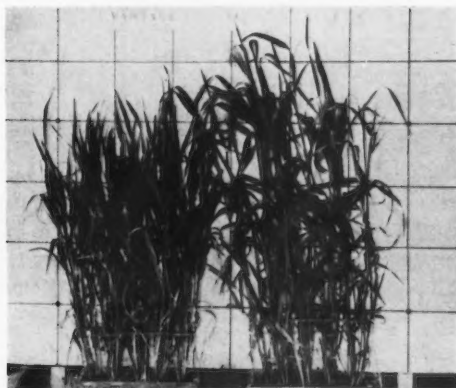
Date of initiation of elongation of the first internode of the first culm was used as an approximation of the time of completion of spikelet differentiation. It has been established that completion of spikelet





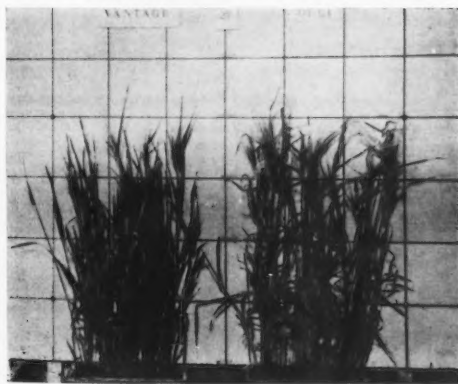
VANTAGE

OLLI



VANTAGE

OLLI



VANTAGE

OLLI

FIGURE 1. Vantage and Olli barley grown under 8-hour (*top*), 16-hour (*centre*) and 24-hour (*bottom*) photoperiods for 80 days at 55°F.

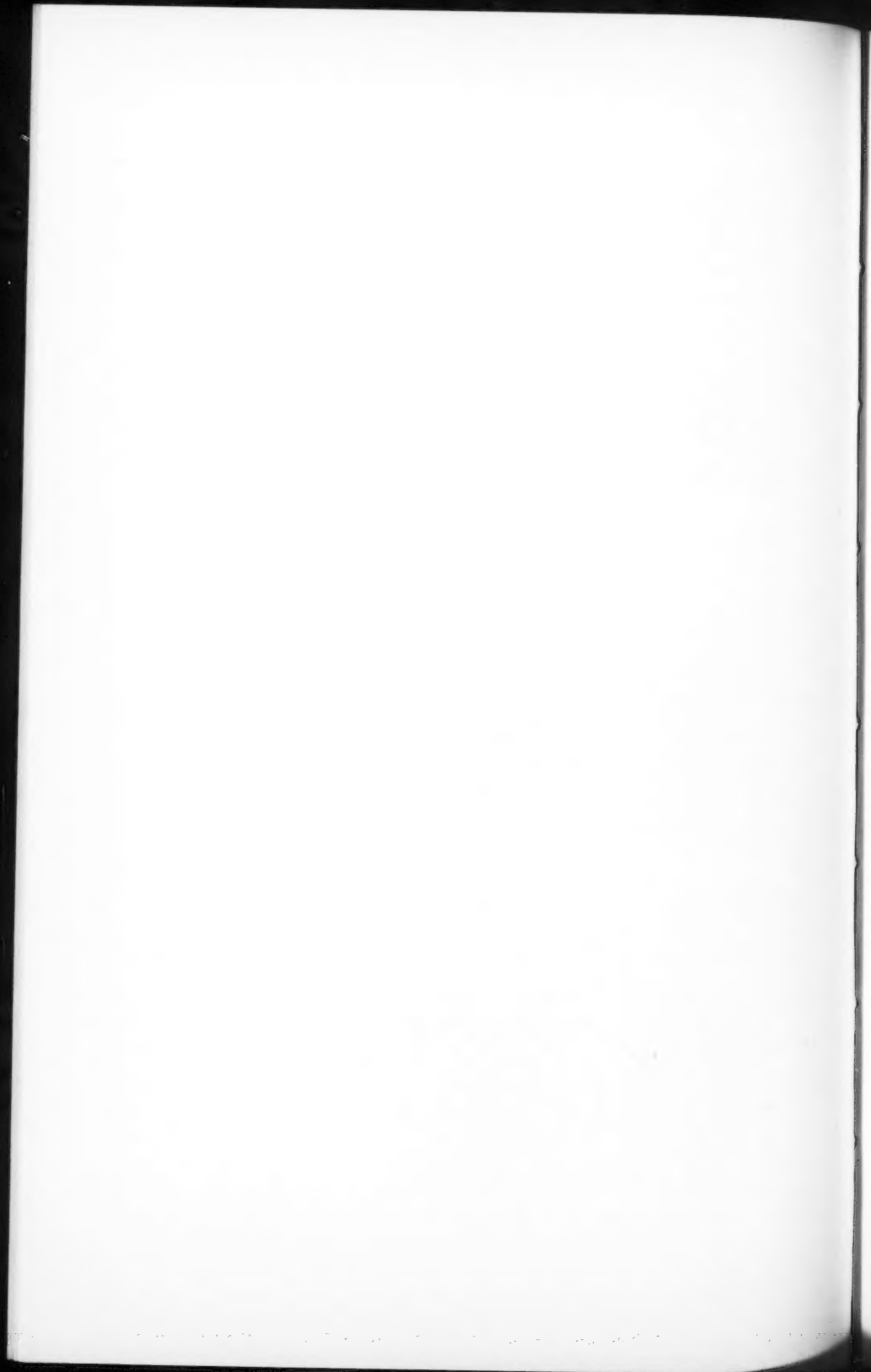


TABLE 2.—AVERAGE NUMBER OF DAYS FROM SEEDING TO INITIATION OF ELONGATION OF THE FIRST INTERNODE OF THE FIRST CULM OF BARLEY

Treatment	Main effect	First order interactions*			
		Variety		Temperature (°F.)	
		O	V	55	75
Variety					
Olli	28.0				
Vantage	57.9 (2.7)				
Temperature (°F.)					
55	44.1	34.0	54.2		
75	41.8 (N.S.)	22.0	61.5		
Photoperiod Stage I (hr.)					
8	70.7	37.2	104.2	68.4	73.0
16	33.0	25.8	40.4	35.0	31.0
24	25.0 (6.9)	21.0	29.0	28.8	21.2

\*Only the first order interactions that are underlined are significant at the 5 per cent level of probability.

NOTE: Least significant differences at the 5 per cent level of probability are shown in parentheses.

differentiation precedes elongation of the first internode (6) and that internode elongation immediately follows the completion of spikelet differentiation\*.

As presented in Table 2 the first internode of the first culm of Olli commenced elongation sooner than that of Vantage under all combinations of photoperiod and temperature. Increase in temperature from 55° to 75°F. advanced the time of internode elongation of Olli under all photoperiods resulting in the 12.0 day average reduction in the number of days from seeding to initiation of internode elongation shown in Table 2. The increase in temperature caused an average delay of 7.3 days in the time of internode elongation for Vantage but the response was conditioned by the photoperiod. Under 8-hour photoperiods there was a 21.7 day delay in time of internode elongation with the increase in temperature, under 16-hour a 2.9 day delay, and under the 24-hour photoperiods a 2.8 day advance in the time of internode elongation. The varieties also responded differently to the increases in photoperiod. Olli commenced internode elongation 8.0 days earlier than Vantage under 24-hour photoperiods but 67.0 days earlier under the 8-hour photoperiods. The influence of photoperiod on the development of Olli and Vantage is illustrated in Figure 1.

Delay in the initiation of elongation of the first internode of Vantage under the 8-hour photoperiod was associated with the development of numerous small, light green, structurally weak leaves. The effect was more pronounced at 75° than at 55°F. Olli produced large, strong plants under the 8-hour photoperiod indicating greater tolerance than Vantage

\*Gfeller, F. The differentiation of the spike or panicle primordium in relation to the development of cereals (mimeographed). Cereal Division, Central Experimental Farm, Ottawa, Ont.

to short photoperiods of the relatively low-intensity, artificial light used. The response of Vantage to the 8-hour photoperiods also suggests that spikelet differentiation and subsequent internode elongation may be delayed but are not completely suppressed by photoperiods adequate for leaf development.

#### *Extent of Leaf Development*

Main effects and first order interactions for the final number and height of leaves, presented in Table 3, provide indices of the extent of development of the leaves associated with the first culm. Vantage produced more leaves than Olli. Leaf number for both varieties was reduced by the increase in temperature, and in photoperiod during Stages I and II. Since leaf initiation is completed by the time of internode elongation (9), the reduction in leaf number during Stage II can only be caused by suppression of development of leaf primordia with the increase in photoperiod.

A positive correlation of 0.964 ( $P.01 = 0.248$ ) between the number of leaves and the number of days from seeding to internode elongation indicates that the number of leaves is determined primarily by the duration of leaf initiation. Variety, photoperiod and temperature appear to have little influence on meristematic activity as expressed by the rate of initiation of successive leaves.

TABLE 3.—AVERAGE NUMBER OF LEAVES AND AVERAGE MAXIMUM HEIGHT OF LEAVES ASSOCIATED WITH THE FIRST CULM OF BARLEY

Treatment	Main effect		First order interactions*									
	No. of leaves	Maximum height of leaves in inches	Variety		Temperature (°F.)		Photoperiod Stage I (hr.)			Photoperiod Stage II (hr.)		
			O	V	55	75	8	16	24	8	16	24
			Maximum height of leaves in inches									
Variety												
Olli	7.1	27.3			30.3	24.3	30.4	28.6	22.8	25.7	28.3	27.9
Vantage	11.5 (0.1)	26.2 (0.7)			28.8	23.7	29.5	26.5	22.7	25.4	26.4	26.8
Temperature (°F.)												
55	9.4	29.6	7.3	11.5			36.7	29.1	22.9	27.5	30.4	30.8
75	9.2 (0.1)	24.0 (0.5)	7.0	11.4			23.2	26.1	22.7	23.6	24.4	24.0
Photoperiod Stage I (hr.)												
8	12.7	30.0	8.4	17.0	13.2	12.2				29.5	30.2	30.2
16	8.2	27.6	6.9	9.5	8.1	8.3				25.7	28.6	28.4
24	7.1 (0.2)	22.8 (0.6)	6.2	8.0	7.0	7.1				21.5	23.2	23.6
Photoperiod Stage II (hr.)												
8	9.5	25.6	7.2	11.8	9.6	9.3	13.0	8.3	7.2			
16	9.3	27.4	7.1	11.6	9.4	9.3	12.8	8.2	7.0			
24	9.1 (0.2)	27.4 (0.6)	7.1	11.2	9.3	9.0	12.4	8.0	7.0			
			Number of leaves									

\*Only the first order interactions that are underlined are significant at the 5 per cent level of probability.  
NOTE: Least significant differences at the 5 per cent level of probability are shown in parentheses.

A positive correlation coefficient of 0.325 ( $P.05 = 0.324$ ) implies little relationship between maximum leaf height and number. This is apparent from Table 3. Olli was significantly taller than Vantage even though it had fewer leaves. Increase in temperature from 55° to 75°F. caused a greater reduction in leaf height than in leaf number. Decrease in photoperiod increased the number of leaves but the influence on height of leaves was partially masked by associated decreases in elongation of the leaf sheaths and blades.

#### Duration of Leaf Development

Positive correlations of 0.937 and 0.867 ( $P.01 = 0.248$ ) for the association number of leaves with the number of days from seeding to emergence of the last leaf and attainment of maximum height of leaves, respectively, (7) indicate that the duration of leaf development is influenced primarily by the number of leaves initiated by the vegetative meristem. However, successive determinations of leaf number and height (7) suggest that the rate of leaf development is accelerated by increases in temperature and photoperiod during Stages I and II. These variations in rate of development must also influence duration of leaf development.

#### Extent of Stem and Head Development

Main effects and first order interactions for length of stem and length of head are presented in Table 4, and for number of florets per head and

TABLE 4.—AVERAGE LENGTH OF STEM AND AVERAGE LENGTH OF HEAD OF THE FIRST CULM OF BARLEY

Treatment	Main effect		First order interactions*									
	Length of stem in inches	Length of head in cm.	Variety		Temperature (°F.)		Photoperiod Stage I (hr.)			Photoperiod Stage II (hr.)		
			O	V	55	75	8	16	24	8	16	24
			Length of stem									
Variety					18.9	14.7	18.4	17.0	15.1	11.9	17.8	20.8
Olli	16.8	3.9			19.5	11.6	19.4	14.8	12.6	11.0	16.9	18.9
Vantage	15.6	3.7										
	(0.5)	(N.S.)										
Temperature (°F.)												
55	19.2	4.5	4.4	4.6			26.5	17.5	13.7	12.9	21.2	23.7
75	13.2	3.1	3.4	2.8			11.2	14.2	14.1	10.0	13.5	16.0
	(0.5)	(0.2)										
Photoperiod Stage I (hr.)												
8	18.9	4.3	4.5	4.1	5.8	2.9				13.9	20.8	22.0
16	15.9	4.1	4.3	4.0	4.7	3.5				10.7	17.0	19.9
24	13.9	3.0	2.9	3.0	3.1	2.8				9.7	14.1	17.8
	(0.6)	(0.2)										
Photoperiod Stage II (hr.)												
8	11.4	2.0	2.4	1.6	1.6	2.3	2.2	2.1	1.6			
16	17.3	4.7	4.8	4.6	5.8	3.5	5.8	5.0	3.3			
24	19.9	4.7	4.6	4.9	6.1	3.3	5.0	5.2	4.0			
	(0.6)	(0.2)										
			Length of head									

\*Only the first order interactions that are underlined are significant at the 5 per cent level of probability.  
Note: Least significant differences at the 5 per cent level of probability are shown in parentheses.

TABLE 5.—AVERAGE NUMBER OF FLORETS PER HEAD AND AVERAGE WEIGHT OF THE STEM AND HEAD OF THE FIRST CULM OF BARLEY

Treatment	Main effect		First order interactions*										
	No. of florets per head	Weight of stem and head in grams	Variety		Temperature (°F.)		Photoperiod Stage I (hr.)			Photoperiod Stage II (hr.)			
			O	V	55	75	8	16	24	8	16	24	
			Number of florets per head										
Variety													
Olli	33.8	0.35				43.3	24.3	39.4	35.9	26.1	23.7	39.4	38.4
Vantage	31.5 (1.7)	0.36 (N.S.)				40.4	22.6	37.7	32.4	24.4	14.9	41.4	38.3
Temperature (°F.)													
55	41.9	0.48	0.46	0.50				55.1	41.9	28.7	21.2	53.2	51.2
75	23.4 (1.3)	0.23 (0.03)	0.24	0.23				22.2	26.4	21.8	17.4	27.5	25.4
Photoperiod Stage I (hr.)													
8	38.6	0.52	0.51	0.53	0.81	0.23					21.6	50.5	44.0
16	34.1	0.35	0.35	0.34	0.41	0.28					20.3	42.2	40.0
24	25.3 (1.6)	0.20 (0.03)	0.19	0.22	0.21	0.19					16.2	28.5	31.2
Photoperiod Stage II (hr.)													
8	19.3	0.16	0.14	0.18	0.20	0.12	0.27	0.13	0.09				
16	40.4	0.42	0.41	0.42	0.56	0.27	0.64	0.41	0.21				
24	38.3 (1.6)	0.49 (0.03)	0.50	0.48	0.67	0.31	0.65	0.51	0.31				
			Weight of stem and head										

\*Only the first order interactions that are underlined are significant at the 5 per cent level of probability.  
NOTE: Least significant differences at the 5 per cent level of probability are shown in parentheses.

weight of stem and head excluding kernels are presented in Table 5. The weight of stem and head excluding kernels must be considered the most precise measure of reproductive development since it is based on the accumulation of structural material. The other three measurements are also suitable criteria of extent of stem and head development as indicated by positive correlation coefficients of 0.939, 0.890 and 0.912 ( $P.01 = 0.248$ ) for the association of weight of stem and head with each of length of stem, length of head and number of florets per head, respectively. This close association is further verified by positive correlation coefficients of 0.849 and 0.951 ( $P.01 = 0.248$ ) for stem length with head length and for head length with number of florets per head, respectively.

There were, however, certain pertinent differences in response. Olli and Vantage did not differ in weight of the stem and head but Olli had a longer stem and produced a greater number of florets per head. Increases in temperature from 55° to 75°F. caused larger average reductions in weight of the stem and head than in the length of stem, length of head, and number of florets per head. There were greater reductions in the head and stem length of Vantage than of Olli with the increase in temperature. The influence of temperature was accentuated by the reductions in photoperiod.

The decrease in extent of stem and head development associated with the increases in photoperiod during Stage I are attributed to reductions

in the duration of spikelet differentiation without corresponding increases in the rate of meristematic activity. These responses parallel those demonstrated for leaf initiation.

If the potential extent of stem and head development is determined during Stage I, the photoperiod during Stage II must influence the degree to which maximum development is realized. Reductions in stem length, head length and weight of stem and head, associated with the decrease in photoperiod during Stage II, parallel the reduction in leaf height recorded in Table 2. The reduction in number of florets with the increase in photoperiod from 16 to 24 hours is also comparable to the reduction in leaf number during Stage II. It is apparent that the photoperiod has a similar influence on the extent of leaf, stem and head development.

TABLE 6.—AVERAGE NUMBER OF DAYS FROM INTERNODE ELONGATION TO HEADING AND FROM HEADING TO MATURITY, PER CENT FERTILE FLORETS AND AVERAGE NUMBER OF KERNELS PER HEAD, AND 1000-KERNEL WEIGHT OF SEED FOR THE FIRST CULM OF BARLEY

Treatment	Days from internode elongation to heading	Days from heading to maturity	Per cent fertile florets	Number of kernels per head	1000-kernel weight of seed in grams
<i>Variety</i>					
Olli	25.6	44.6	33.1	14.5	31.3
					**
Vantage	30.3	45.3	31.9	13.1	23.8
<i>Temperature (°F.)</i>					
55	31.9	55.6	38.9	22.1	26.8
	**	**	**	**	
75	20.4	22.6	15.5	3.2	28.8
<i>Photoperiod Stage I (hr.)</i>					
8	34.4	46.0	38.6	20.6	33.5
	*		**	*	
16	27.2	53.7	30.6	11.6	32.3
	**	**	**	**	
24	22.0	58.3	21.8	5.8	27.2
<i>Photoperiod Stage II (hr.)</i>					
8	—	—	—	—	—
16	33.0	46.0	14.6	8.2	25.2
	**		**	**	
24	23.8	47.2	44.0	19.4	28.7
<i>Photoperiod Stage III (hr.)</i>					
8		45.5	27.6	14.6	25.6
		**			
16		40.5	29.5	15.3	28.4
		**			
24		35.7	30.0	15.6	30.8

NOTE: Because of missing values, the same data were not always used for calculating response to various treatments. The t-test for paired data was used to determine the significance of differences in average response to levels of each treatment. Single or double asterisks between averages indicate that the differences between treatments exceed the 5 or 1 per cent levels of probability, respectively.



### *Duration of Stem and Head Development*

All possible comparisons of the number of days from internode elongation to heading are presented in Table 6. There was a positive correlation of 0.680 ( $P.05 = 0.316$ ) for the association of the number of days from internode elongation to heading with the weight of stem and head.

The similarity in the average number of days required for Olli and Vantage to develop from internode elongation to heading, and in the extent of stem and head development, indicate no varietal difference in the rate of development. The reductions in the number of days from internode elongation to heading caused by increases in temperature, and photoperiod during Stage I, correspond to the reductions in the extent of development of the stem and head. The comparisons reflect the influence of the degree of differentiation of the stem and head on both the extent and the time required for completion of development.

Plants grown under the 8-hour photoperiods during Stage II did not head, irrespective of temperature or photoperiod during Stage I. The reduced stem elongation was accompanied by poor head development and the eventual death of the last leaf, usually the shot blade. Increase in photoperiod from 16 to 24 hours during Stage II caused the characteristic reduction in the time required for the first culm to progress from internode elongation to heading.

### *Extent and Duration of Development Following Heading*

Number of days from heading to maturity, per cent fertile florets, number of kernels per head and 1000-kernel weight of seed were used to assess the responses of Olli and Vantage to temperature and photoperiod during heading to maturity of the first culm. Averages for the main effects are presented in Table 6. Data were limited to the plants that headed and were transferred to the photoperiods specified for Stage III. Data on the number of days from heading to maturity are further restricted to the treatments that produced fertile plants.

At 75°F. the first culm of Olli was fertile only when plants were grown under 8-, 16- or 24-hour photoperiods during Stage I and transferred to 24-hour photoperiods during Stage II. At 55°F. Olli was also fertile under the 24-hour photoperiods during Stage II, and when the plants were transferred from 8- or 16-hour photoperiods during Stage I to 16-hour photoperiods during Stage II. At 75°F. the first culm of Vantage was fertile when plants were grown under 16- or 24-hour photoperiods during Stages I and II, and when transferred from 16-hour photoperiods during Stage I to 24-hour photoperiods during Stage II. At 55°F. Vantage produced fertile heads when the plants were grown under 8-, 16- or 24-hour photoperiods during Stage I and transferred to 24-hour photoperiods during Stage II, and when grown under 8-hour photoperiods during Stage I and transferred to 16-hour photoperiods during Stage II.

The number of days from heading to maturity emphasizes further the essential similarity in the development of the first culms of Olli and Vantage. The response to temperature followed the pattern established for stem and head development. Increase in photoperiod during Stage I caused a

reduction in stem and head development but an increase in the number of days from heading to maturity, while increase in photoperiod during Stage II promoted stem and head development but did not affect the time required for development. Increase in photoperiod during Stage III accelerated development.

A positive correlation of 0.797 ( $P.01 = 0.331$ ) between number of florets and number of kernels per head indicates some variation in fertility. As shown in Table 6, Olli and Vantage did not differ significantly in average fertility. The decreases in number of florets, shown in Table 4, and in fertility associated with the increases in temperature and in photoperiod during Stage I caused large reductions in the number of kernels per head. The increase in photoperiod from 16 to 24 hours during Stage II reduced the number of florets but more than tripled fertility resulting in a net increase in the number of kernels per head. It is worthy of special note that the number of kernels and the fertility were influenced by the photoperiod during Stages I and II but not during Stage III when fertilization was observed to occur under most treatments.

A positive correlation of 0.983 ( $P.01 = 0.331$ ) indicates a close association between the number of kernels and the weight of seed per head. As shown in Table 6, the kernels of Olli were heavier than those of Vantage whereas in the field the reverse is usually true. The 1000-kernel weights were not influenced significantly by temperature or photoperiod.

TABLE 7.—AVERAGE NUMBER OF DAYS FROM ELONGATION OF THE FIRST INTERNODE OF THE FIRST CULM TO INITIATION OF THE FIRST TILLER OF OLLI AND VANTAGE BARLEY

Treatment	Main effect		First order interactions*				
	Olli	Vantage	Temperature (°F.)		Photoperiod Stage I (hr.)		
			55	75	8	16	24
Variety	21.0	-5.3	<div style="display: flex; align-items: center; justify-content: center;"> <div style="writing-mode: vertical-rl; transform: rotate(180deg);"> Vantage </div> <div style="border-left: 1px dashed black; border-right: 1px dashed black; height: 100%; margin: 0 10px;"></div> <div style="writing-mode: vertical-rl; transform: rotate(180deg);"> Olli </div> </div>				
Temperature (°F.)							
55	17.4	-15.8					
75	24.7 (1.2)	5.2 (2.7)					
Photoperiod Stage I (hr.)							
8	27.4	-11.1					
16	19.2	-5.6					
24	16.5 (1.5)	0.8 (3.3)					
Photoperiod Stage II (hr.)							
8	30.1						
16	19.8						
24	13.2 (1.5)						

\*Only the first order interactions that are underlined are significant at the 5 per cent level of probability.  
NOTE: Least significant differences at the 5 per cent level of probability are shown in parentheses.

## PHENOLOGY AND MORPHOLOGY OF TILLERS

*Initiation of Tillering*

Main effects and first order interactions for the average number of days from elongation of the first internode to initiation of the first tiller are presented in Table 7. Tillering of Olli did not commence until after termination of leaf development of the first culm but, depending on treatment, Vantage commenced tillering both before and after. Because of this, the varieties were analysed separately for response to temperature and photoperiod. The earlier tillering of Vantage relative to time of internode elongation of the first culm may be associated with the more extensive leaf development. The influence of temperature on initiation of tillering tends to verify this supposition.

Increases in photoperiod during Stage I caused successive delays in the initiation of tillering of Vantage at 55°F. The delay in initiation

TABLE 8.—AVERAGE NUMBER OF DAYS FROM INITIATION TO TERMINATION OF TILLERING, AND THE NUMBER AND CHARACTERISTICS OF TILLERS OF BARLEY

Treatment	Days from initiation to termination of tillering	Number of tillers					Weight of stem and head in grams	Number of kernels per head	Weight of seed per plant in grams
		Total	Fertile	Sterile	Immature	Rudimentary			
<i>Variety</i>									
Olli	36.5	4.5	0.7	1.3	1.4	1.1	0.66	8.0	0.18
Vantage	36.6	4.2	0.6	1.5	1.1	1.0	0.73	8.1	0.2
<i>Temperature (°F.)</i>									
55	53.2 **	6.0 **	0.8 **	2.3 **	2.0 **	1.0 **	1.07 **	9.0 **	0.22 **
75	18.1	2.7	0.3	0.6	0.5	1.4	0.18	2.3	0.04
<i>Photoperiod Stage I (hr.)</i>									
8	31.0 *	3.7 **	0.6	1.0 *	0.9 **	1.2	0.66 *	13.0	0.30
16	39.9	5.4 **	0.8	1.8	1.6	1.2	0.87 **	8.4 *	0.18
24	38.0	4.9	0.7	1.8	1.5	1.0	0.74	5.3	0.16
<i>Photoperiod Stage II (hr.)</i>									
8	—	—	—	—	—	—	—	—	—
16	35.0 *	4.2	0.4 **	1.5 **	1.1	1.1	0.71 **	6.1 *	0.10 **
24	32.4	4.2	0.7	1.1	1.2	1.1	0.58	8.9	0.25
<i>Photoperiod Stage III (hr.)</i>									
8	35.7	4.3	0.3 **	1.3	1.4 **	1.3 *	0.49 **	6.0	0.07 **
16	35.3	4.2	0.6	1.5	1.1	1.0	0.66 **	6.7 **	0.14 **
24	33.6	4.3	0.9	1.2	1.1	1.1	0.79	9.8	0.32

NOTE: Because of missing values, the same data were not always used for calculating response to various treatments. The t-test for paired data was used to determine the significance of differences in average response to levels of each treatment. Single or double asterisks between averages indicate that the differences between treatments exceed the 5 or 1 per cent levels of probability, respectively.

of tillering with the 8-hour photoperiods at 75°F. is probably associated with the retarded leaf development. Acceleration of the initiation of tillering of Olli with the increase in photoperiod is opposed to the response of Vantage. Thus, Olli and Vantage not only differ in the relative time of tiller initiation but respond differently to photoperiod.

#### *Duration of Tillering*

Average number of days from initiation to termination of tillering are presented in Table 8. Although Olli and Vantage commenced tillering at different stages in the development of the first culm, they did not differ in the average number of days from initiation to termination of tillering. Duration of tillering must then be independent of the time of initiation of tillering.

Reduction in duration of tillering with the increase in temperature from 55° to 75°F. was more extreme than the corresponding reduction in time required for the development of the first culm. Duration of tillering was influenced by photoperiod but the trends are not as definite as those for variety and temperature. The data suggest an increase in the duration of tillering with the increase in photoperiod from 8 to 16 hours and a reduction with a further increase to 24 hours.

#### *Number and Characteristics of Tillers*

Extent of tiller development was based on the total number of tillers produced and on the stage of development of the tillers at the time of termination of development of the first culm. The total number of tillers and the number of fertile, sterile, immature and rudimentary tillers on each plant are presented in Table 8. A positive correlation of 0.904 ( $P.01 = 0.316$ ) indicates a close association between duration of tillering and the total number of tillers. The apparent limited influence of variety, photoperiod and temperature on the rate of tiller initiation is consistent with the stability of rate of initiation of the leaves and florets associated with the development of the first culm.

The similarity of Olli and Vantage in duration of tillering and number of tillers, and the large difference between the varieties in duration and extent of development of leaves on the first culm, suggest that leaf development on the first culm and tillering are independent functions. The decrease in number of tillers with the increase in temperature is associated with the reduction in duration of tillering. The significant increase in the number of rudimentary tillers reflects the delay of tiller initiation with increase in temperature.

None of the tillers of Olli and only a few for Vantage emerged prior to internode elongation of the first culm but, as shown in Table 8, photoperiod influenced total number of tillers only during this stage of development. Although Olli and Vantage varied markedly in the time of initiation of tillering relative to internode elongation of the first culm, the number of active tiller initials was determined apparently prior to spikelet differentiation.

A significantly greater number of tillers was produced by plants grown under the 16-hour photoperiods than by plants under 8- or 24-hour

photoperiods during Stage I. Photoperiod during Stages II and III did not influence the total number of tillers but increase in photoperiod during Stage II increased the number of fertile and decreased the number of sterile tillers. Increase in photoperiod during Stage III increased the number of fertile and decreased the number of immature and rudimentary tillers. This suggests an increase in rate and possibly an associated reduction in extent of development of tillers with increase in photoperiod.

Average weight per plant of the stems and head, excluding kernels, was used as an index of the total stem and head development of the tillers. Average number of kernels per head and average weight of kernels per plant were used as indices of kernel development of the fertile tillers. The data are presented in Table 8.

A correlation coefficient of 0.813 ( $P.01 = 0.316$ ) indicates a close association between duration of tillering and weight of the stem and head. Increase in the photoperiod from 16 to 24 hours during Stage II increased the number of fertile tillers but caused a significant decrease in the average weight of the stems and heads produced by the tillers. Since spikelet differentiation for many of the tillers occurred during Stage II the response is coincident with that of the first culm to photoperiod during Stage I. In like manner, the increase in the stem and head weight of the tillers with increase in photoperiod during Stage III is similar to the influence of photoperiod on the first culm during Stage II. It is apparent that the photoperiod had a similar influence on the extent of stem, head and kernel development of the first culm, and of the successive tillers.

### CONCLUSIONS

The environment provided by the growth chambers and artificial growth medium was adequate for the growth of Olli and Vantage spring barley and provided precise control of photoperiod and temperature. The relatively low intensity of artificial illumination and the rigid control of the artificial climate may have caused growth responses, such as reduced fertility, that would not be obtained in the field.

Increase in photoperiod reduced the number of days required for completion of the successive stages of development of the first culm. During seeding to internode elongation the accelerated development was caused primarily by the development of fewer leaves before transition of the apical meristem to the reproductive state. Following internode elongation, increase in photoperiod also caused more extensive development of the first culm indicating increases in the rate of accumulation of structural material.

Increase in photoperiod during seeding to internode elongation caused earlier initiation of tillering relative to the development of the first culm but there were strong interactions with variety and temperature. Duration of initiation of tillering tended to increase with increase in photoperiod from 8 to 16 hours but to decrease with further increase to 24 hours. Increase in photoperiod following elongation of the first culm did not influence the total number of tillers produced but caused increases in the number of

fertile and corresponding decreases in the number of sterile, immature and rudimentary tillers. The influence of photoperiod on the structural development of the tillers was essentially similar to the influence on the first culm.

Increase in temperature from 55° to 75°F. accelerated plant development and caused reductions in plant size. The reduction in size was caused by decreases in both the number and development of individual plant parts. There were strong interactions of photoperiod and temperature, the influence of temperature being reduced with increase in photoperiod.

Plant response was usually conditioned by photoperiod during both the immediate and prior stages of development. There were two notable exceptions. The number of kernels on the main culm was influenced by photoperiod during heading, but not by photoperiod following heading. Although tiller emergence normally commenced after internode elongation, duration of tiller initiation and the total number of tillers were only influenced by photoperiod prior to internode elongation.

The relative performance of Olli and Vantage was not the same under all photoperiods. The 8-hour photoperiods approached the minimum for leaf development and subsequent spikelet differentiation of the first culm of Vantage but were adequate for normal growth of Olli. As illustrated in Figure 1, the varieties became progressively more alike in duration and extent of growth as the photoperiod was increased from 8 to 16 to 24 hours.

Certain developmental aspects of the two varieties are of practical significance. The later maturity of Vantage was caused primarily by prolongation of leaf initiation but the greater duration of this stage of development did not contribute to greater stem and head development. This relationship suggests that it might be possible to select for the reduced leaf development associated with earlier maturity without causing reductions in yield potential. Selection for earlier initiation of tillering should also be possible without causing a reduction in tillering potential. However, it might be difficult to combine early tillering and reduced leaf development.

The high correlation for number of leaves and number of days from seeding to internode elongation indicates that either of these measurements can be used as an index of duration and extent of leaf development. For certain varieties these measurements could also provide an indication of earliness of maturity. Stem length, head length, and number of florets per head appear to be equally satisfactory measurements of extent of stem and head development prior to kernel formation but are not accurate indices of duration of reproductive development. There is also a close relationship between the number of tillers and duration of tillering.

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# THE USE OF CEREAL GRAINS AS COMPANION CROPS IN DRYLAND FORAGE CROP ESTABLISHMENT

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## ABSTRACT

The effect of wheat, oats, barley, and spring rye as companion crops on the establishment of a perennial forage crop mixture consisting of crested wheatgrass, brome, and alfalfa under arid conditions compared to no companion crop was studied at Swift Current, Saskatchewan. Cereal companion crops reduced the vigour, stand, and subsequent early forage yields of the grass-alfalfa mixtures, but less so if the cereal crop and the forage crop were seeded separately at right-angles to one another. The method of harvesting the cereal companion crop also influenced the performance of the subsequent forage crop. Cutting the cereal crops at a height of 8 inches or more for grain resulted in better grass-alfalfa stands and yields than was obtained when the cereal crops were mowed at a 2-inch height for hay. The effect of kind of cereal grain on performance of the perennial forage differed little. Wider row spacings for the cross-seeded companion crops also resulted in a better stand and yield of the grass-alfalfa crop.

## INTRODUCTION

The use of cereal grains as companion or nurse crops in perennial forage crop seedings has been a long established agricultural practice. Reports on agricultural cropping culture during the early history of settlement in North America make numerous references to the practice of seeding perennial forage grasses and legumes together with cereal grains. A common method of seeding the two crops was to seed the grain first with a seeder and then broadcast the forage crop over the same area later. It would appear that the early farmers and agronomists considered the use of the companion crop to be essential in successful forage crop establishment. Even today most farmers throughout the Prairies seed perennial forage crops with a cereal companion crop. The most common method is to mix the two crops and seed them together.

With the extension of settlement and cultivation into the more arid parts of North America, the problems in small seeded forage crop establishment increased. Failures or near failures to establish stands occurred more frequently, particularly in the arid regions and especially when seeded with a cereal companion crop. However, a long-standing practice which has been credited with certain advantages is not soon discarded.

The studies discussed in this paper were started in 1950 to gain more information on the effect of companion crops on establishment of grasses and legumes in an arid climate. Considerations included the influence of cross-seeding the companion crop vs. mixed seeding, methods of harvesting the cereal crop, effect of row spacing, and choice of cereal grain. The influence of these treatment factors was measured by determining their effect on perennial forage crop stand and subsequent yield. In addition, the hay and grain yields obtained from the annual cereal companion crops themselves were measured. Tests were conducted from 1950 to 1954,

inclusive, at Swift Current, Saskatchewan, on summerfallowed land which had been cropped according to a grain-summerfallow rotation for 30 years or more.

#### LITERATURE REVIEW

In reviewing companion crop work the main advantage offered was the "nurse crop" concept whereby the tougher, faster growing cereal grain provided the necessary protection for the small, slower growing grass and legume seedlings (2, 4, 5, 7, 8, 9, 10, 12). This "nurse crop" concept has been described as protection against wind and water erosion, soil drifting, animal grazing, soil crusting, weed control, and even too intense sunlight. Not the least important consideration for using cereal companion crops has been the added hay or grain crop obtained in the seeding year which might otherwise have been forfeited if a companion crop were not used (1, 4, 11, 12, 14).

On the other hand, the disappointing forage crop stands resulting from the use of companion crops have been readily acknowledged by many agricultural workers (1, 2, 4, 5, 11, 12, 13, 14). These reports express a generally agreed upon theme that, when moisture is the most serious limiting factor in successful crop establishment, one cannot expect to simultaneously grow out both a cereal crop and a forage crop to fruitful yield. Progress reports and annual reports of Experimental Farms throughout the Prairies point out the common deleterious effect of companion crops. Furthermore, there have been, and are, standing recommendations (7, 8) that with certain perennial forage crops, notably legumes in pure seedings (6, 7, 10), a cereal companion crop should not be used. All of the foregoing references to the use of companion crops confined the practice to mixed seedings of the forage and cereal seeds. Also, most of the forage crop seedings with companion crops were made with drills in row spacings of 6 to 8 inches. If the forage crop was not mixed with the cereal grain it was broadcast, but the cereal companion crop was still seeded in the standard row width.

In 1935 and 1936 Fryer wrote (5, 7, 8) that the use of "nurse crops" in Alberta should be limited to areas of good moisture and then at reduced rates. Brown (1) in 1931 also pointed out the important limitations of "nurse crop" use in Manitoba and advocated reduced seeding rates for the cereal used. In 1946 the effect of wider row spaces for the cereal companion crop was reported on by Harper (11). He found that stands of sweet clover in the Southern Great Plains area were greatly improved by using 14-inch spaces between the cereal rows, particularly during dry seasons.

As early as 1935, Kirk of Saskatchewan (12) reported that excellent results were obtained from cross-seeding the forage seed after the cereal companion crop had been seeded in one direction by itself. About this same time a report from the Experimental Farm at Scott, Saskatchewan (15) makes a reference to the "promising indications" from cross-seeding companion crops. Again, in 1943, English and Mather (2) advise the cross-seeding principle for Alberta. However, none of these reports (2, 12, 15) was substantiated by published experimental data.

### MATERIALS AND METHODS

The study was divided into two separate but adjacent tests because of the difficulty of cross-seeding long, narrow plots which would have been randomly located had only one large study been used. The two-test division therefore was arranged into Test 1 (where the forage crop mixture and the cereal companion crops were mixed and seeded together), and Test 2 (where the forage mixture was seeded at right angles to the cereal companion grain). The size of all plots in both tests was 17 by 40 feet. Seeding was done with a 16-run double disk wheel drill, equipped with removable clamp-on depth control attachments. Cereal companion crops used in this study were: wheat,  $\frac{1}{2}$  bushel per acre, oats at 1 bushel, barley at  $\frac{3}{4}$  bushel, and spring rye at  $\frac{1}{2}$  bushel, while a mixture of crested wheat-grass, brome, and alfalfa constituted the forage crop in a 2:4:2 pounds per acre ratio.

#### Test 1

*Forage and cereal crops were mixed and seeded together.* Plots were arranged in a split-plot design with four replications. Main plots were kind of cereal mixed with the forage seed while method of harvesting the cereals made up the subplots. Only 12-inch row spacings were used in this test by seeding through every second drill opening. Depth control attachments maintained the seeding depth of the mixed crops to approximately 1 inch. When the cereal companion crops were at a milk to soft dough stage of maturity, one longitudinal half of each plot was cut with a mower to determine annual cereal hay yields. The other longitudinal half of each plot was left to mature seed which was harvested so as to leave an 8-inch stubble.

#### Test 2

*The forage mixture was seeded separately at right angles to the cereal companion crop rows.* Plots were arranged in a split plot design with four replications. Main plots were kind of cereal, each in 6- or 12-inch spaced rows, while harvesting method constituted the subplots. In this test the plots were first seeded separately (with depth controls removed) to the appropriate cereal grain in either a 6- or 12-inch spaced row width at a 3-inch depth. The forage crop mixture was then seeded crosswise over the entire width of the test area. Row spacings for the forage crop was 12 inches and seeding depth was maintained to 1 inch by using the depth control attachments. Each plot was split longitudinally as described for Test 1 with one-half being harvested for cereal hay while the remaining half was harvested for grain.

Each spring, following the establishment year, the split plots within both tests were sampled with a point quadrat frame to determine per cent basal ground cover of the forage plants. These values were the ones used to express stands. First-year grass-legume hay cuts were also taken on a split-plot basis. During the second crop year hay yields were again taken on the split-plot basis after which the tests were ploughed up. Thus, the measurements included hay and grain yields of the cereal crops in the establishment year, the stand and hay yield of the forage in its first crop year, and the stand and yield of forage in the second forage crop year.

TABLE 1.—STAND OF FORAGE CROP IN THE FIRST CROP YEAR FOLLOWING ESTABLISHMENT AS AFFECTED BY HARVEST METHOD OF COMPANION CROP

Cereal companion crop used	Percentage basal ground cover												
	Test 1—Forage and companion crop together						Test 2—Forage and companion crop cross seeded						
	When companion crop harvested as hay			When companion crop harvested as grain			When companion crop harvested as hay			When companion crop harvested as grain			
	1951	1952	1953	3-year av.	1951	1952	1953	3-year av.	1951	1952	1953	3-year av.	1951
<i>Alfalfa Portion</i>													
Wheat	0.13	1.17	0.85	0.72	0.39	1.50	1.42	1.35	1.17	0.88	1.82	1.80	1.50
Oats	0.16	0.90	0.77	0.61	0.41	0.77	1.18	0.79	1.04	0.73	1.44	1.55	1.24
Barley	0.08	1.00	0.55	0.54	0.45	1.57	1.35	1.12	1.17	0.72	1.52	1.45	1.24
Rye	0.11	0.94	0.64	0.56	0.49	1.30	1.41	1.07	0.89	0.80	1.46	1.61	1.29
Check	0.86	1.80	1.75	1.47	0.86	1.80	1.75	1.47	1.46	0.85	1.67	1.85	1.46
L.S.D. (P = .05)	0.27	0.76	0.45		0.31	0.43	N.S.			N.S.	N.S.	N.S.	
S.E.M. %	11.01	7.91	6.85		8.27	9.08	11.73			6.41	5.71	7.90	
<i>Grass Portion</i>													
Wheat	0.65	2.11	3.03	1.93	0.91	5.03	4.15	3.36	3.97	1.68	6.10	5.16	4.31
Oats	1.30	2.33	4.27	2.63	1.40	2.95	3.59	2.65	3.52	1.62	5.35	4.84	3.94
Barley	0.93	1.90	2.78	1.87	1.17	2.43	3.26	2.29	3.45	2.25	4.55	5.27	4.02
Rye	1.16	1.56	3.02	1.91	1.14	3.07	3.48	2.56	3.44	2.41	4.72	5.02	4.05
Check	2.87	5.03	5.16	4.35	2.87	5.03	5.16	4.35	5.06	3.01	6.70	5.47	5.06
L.S.D. (P = .05)	0.77	1.45	1.03		0.90	2.10	1.18			1.32	N.S.	N.S.	
S.E.M. %	9.21	6.39	7.82		8.81	7.25	6.35			7.20	4.14	4.76	

TABLE 2.—STAND OF FORAGE CROP IN THE SECOND CROP YEAR FOLLOWING ESTABLISHMENT AS AFFECTED BY HARVEST METHOD OF COMPANION CROP

Cereal companion crop used	Percentage basal ground cover											
	Test 1—Forage and companion crop together						Test 2—Forage and companion crop cross seeded					
	Companion crop harvested as hay			Companion crop harvested as grain			Companion crop Harvested as hay			Companion crop harvested as grain		
	1952	1953	2-year av.	1952	1953	2-year av.	1952	1953	2-year av.	1952	1953	2-year av.
	Alfalfa Portion											
Wheat	0.97	2.15	1.56	1.42	2.96	2.19	2.64	2.97	2.80	3.62	4.61	4.12
Oats	1.50	1.96	1.73	2.22	3.13	2.68	2.12	3.05	2.58	3.30	4.27	3.78
Barley	1.42	2.61	2.02	1.70	3.17	2.44	2.51	2.77	2.41	3.50	4.04	3.77
Rye	1.02	2.38	1.70	2.00	3.45	2.72	2.03	2.52	2.38	3.87	4.07	3.97
Check	3.40	4.19		3.80	4.19		3.80	3.42	3.90	3.42	4.37	3.90
L.S.D. (P = .05)	1.12	1.73		1.07	0.76		N.S.	1.83		N.S.	N.S.	
S.E.M. %	8.31	9.12		7.35	7.19		5.79	6.92		5.97	6.10	
	Grass Portion											
Wheat	5.07	5.89	5.48	5.40	7.23	6.32	10.25	11.15	10.70	9.54	11.63	10.08
Oats	5.85	5.88	5.82	5.40	8.77	7.08	9.26	13.76	11.51	6.68	10.96	8.82
Barley	7.92	6.05	6.98	6.42	5.11	5.76	8.86	10.45	9.66	8.75	12.52	10.64
Rye	6.33	6.07	6.20	5.10	7.22	6.16	9.12	12.82	10.97	9.16	10.61	9.88
Check	10.75	12.36		11.50	12.36		11.12	12.82	11.93	11.12	12.74	11.93
L.S.D. (P = .05)	2.87	3.15		3.70	2.91		N.S.	N.S.		2.88	N.S.	
S.E.M. %	8.11	7.77		9.10	6.58		6.75	6.10		9.31	7.05	

## RESULTS

There were actually two main criteria to be considered in examining the results and assessing the merits of the companion crop treatments. These were first, the stands and yields of the forage crop following establishment; and second, the performance of the companion crop itself measured in terms of yield returns.

Although the experiments were laid out according to a split-plot design and the original statistical analyses were made on this basis, it was found that the data could be interpreted more clearly if simple variance analyses were applied to data from various parts of the tests. The results from the original split-plot analysis indicated, with very few exceptions, the lack of significance for the interaction cereal crop times harvesting methods for both yield and stand measurements. For this reason the results are presented in a series of simple tables each of which contains pertinent data from the two tests.

### *Forage Crop Performance*

Forage crop stands were determined in the first forage crop year for 1951, 1952, and 1953 (Table 1), and in the second crop year for 1952 and 1953 (Table 2). In both tests in most years the first-year crop cover of the alfalfa and the grass component was usually significantly less when established with a cereal companion crop than when grown without a companion crop (Table 1). Cross-seeded companion crops reduced forage cover to a lesser extent than did companion crops which were seeded in the same drill row with the forage mixture. The cover of the forage components in both tests was reduced most when the cereal companion crop was mowed for hay as compared to when it was harvested for grain. It should be noted that, when companion crops were cross-seeded and harvested for grain, the resulting stand of both alfalfa and grass was only slightly less than the stand obtained when no companion crop was used.

In the arid regions of the Canadian Prairies the percentage basal ground cover of perennial forage crops does not usually exceed 5 per cent during the seedling year. Even following the second growing year the basal ground cover is most commonly confined to the 10 to 15 per cent range. This will explain the seemingly low range of cover values shown in Tables 1 and 2, particularly to those readers from other cropping areas who are not accustomed to such thin stands.

Table 2 shows the stand of the forage components in the second forage crop year. Although a substantial natural increase in cover from the first to second crop year was recorded, there still existed a marked difference in the stand because of companion crop influence. However, the forage stand on cross-seeded treatments where the companion crop had been harvested for grain was almost on a par with forage stands where no companion crop was used.

Differences in grass or legume stand due to kind of cereal were small and in most cases not significant.

Table 3 summarizes the dry matter forage crop hay yields obtained from both tests in the first and second crop years. Reduced forage crop



TABLE 3.—SUBSEQUENT FORAGE CROP HAY YIELDS AS AFFECTED BY METHOD OF SEEDING AND METHOD OF COMPANION CROP HARVEST

Cereal companion crop used	Dry matter forage yield—tons per acre														
	Test 1—Forage and companion crop seeded together					Test 2—Forage and companion crop cross seeded									
	Companion crop harvested as hay					Companion crop harvested as grain									
	1951	1952	1953	1954	3-year av.	1951	1952	1953	1954	3-year av.					
<i>First Forage Crop Year</i>															
Wheat	0.08	0.18	0.55	0.27	0.16	0.32	0.81	0.43	0.16	0.20	0.77	0.26	0.24	0.84	0.45
Oats	0.07	0.12	0.46	0.25	0.18	0.28	0.68	0.38	0.12	0.19	0.61	0.20	0.24	0.74	0.39
Barley	0.09	0.16	0.64	0.30	0.13	0.26	0.73	0.38	0.14	0.17	0.66	0.32	0.19	0.80	0.41
Rye	0.11	0.10	0.46	0.22	0.17	0.17	0.72	0.35	0.17	0.14	0.71	0.26	0.18	0.74	0.39
Check	0.40	0.31	1.07	0.59	0.40	0.31	1.07	0.59	0.35	0.28	1.06	0.35	0.28	1.06	0.56
L.S.D. (P = .05)	0.11	0.12	0.31		0.13	N.S.	0.20		0.10	0.09	0.22	0.08	N.S.	0.30	
S.E.M. %	9.15	8.08	6.61		8.78	5.44	6.30		8.31	7.93	7.75	7.12	10.41	8.11	
<i>Second Forage Crop Year</i>															
Wheat	0.26	0.62	0.85	0.58	0.38	0.75	0.93	0.69	0.33	0.94	1.00	0.76	0.34	1.10	1.12
Oats	0.29	0.71	0.81	0.60	0.52	0.69	0.96	0.72	0.33	0.78	0.93	0.68	0.44	1.00	1.06
Barley	0.26	0.66	0.74	0.55	0.44	0.80	0.87	0.70	0.32	0.81	0.97	0.70	0.38	0.95	0.98
Rye	0.28	0.74	0.70	0.57	0.40	0.81	0.71	0.73	0.34	0.89	1.10	0.78	0.41	0.92	1.07
Check	0.49	1.09	1.07	0.88	0.49	1.09	1.07	0.88	0.53	1.06	1.10	0.90	0.53	1.06	1.10
L.S.D. (P = .05)	0.19	0.23	N.S.		N.S.	0.16	N.S.		0.14	N.S.	N.S.		N.S.	N.S.	
	7.03	6.84	9.35		8.31	7.76	7.17		6.26	7.34	5.17		6.93	5.29	5.76



TABLE 4.—SUBSEQUENT FORAGE CROP HAY YIELDS AS AFFECTED BY ROW SPACING OF COMPANION CROP (TEST 2, CROSS SEEDING)

Cereal companion crop used	D.M. Forage yields—tons per acre									
	6-inch row spacing companion crop					12-inch row spacing companion crop				
	1951	1952	1953	1954	4-year av.	1951	1952	1953	1954	4-year av.
<i>First Forage Crop Year</i>										
Wheat	0.18	0.18	0.62	0.49	0.37	0.23	0.23	0.89	0.61	0.49
Oats	0.13	0.18	0.61	0.39	0.33	0.19	0.24	0.68	0.64	0.44
Barley	0.14	0.15	0.64	0.44	0.34	0.20	0.20	0.76	0.62	0.44
Rye	0.21	0.16	0.66	0.77	0.34	0.23	0.16	0.76	0.77	0.38
Check	0.35	0.28	1.06		0.62	0.35	0.28	1.06		0.62
L.S.D. ( $P = .05$ )	0.09	0.08	0.22	0.29		0.10	N.S.	0.17	N.S.	
S.E.M. %	3.78	4.16	5.58	6.78		4.19	5.73	6.13	4.96	
<i>Second Forage Crop Year</i>										
Wheat	0.29	0.70	0.96	0.65	0.66	0.38	0.36	0.92	1.07	0.79
Oats	0.36	0.64	1.06	0.69	0.66	0.36	0.74	0.74	1.02	0.71
Barley	0.31	0.72	1.02	0.68	0.66	0.33	0.74	0.85	1.09	0.72
Rye	0.31	0.62	0.94	0.62	0.90	0.37	0.85	1.04	1.04	0.75
Check	0.53	1.06	1.10			0.53	0.53	1.06	1.10	0.90
L.S.D. ( $P = .05$ )	0.14	0.23	N.S.			0.16	N.S.	N.S.	N.S.	
S.E.M. %	5.92	5.26	6.10			5.37	6.10	6.10	4.93	

TABLE 5.—CEREAL COMPANION CROP HAY AND GRAIN YIELDS—SEEDED CROSSWISE AND WITH FORAGE CROP

Cereal companion crop	Test 1—Forage and companion crop seeded together						Test 2—Forage and companion crop cross seeded					
	1950	1951	1952	1953	1954	5-year av.	1950	1951	1952	1953	1954	5-year av.
	<i>D.M. hay yields—tons per acre</i>											
Wheat	0.74	1.09	2.14	1.41	0.76	1.23	0.92	1.01	1.86	1.41	0.92	1.22
Oats	1.01	1.40	2.51	2.06	0.98	1.59	1.16	1.61	2.05	1.98	1.27	1.66
Barley	0.75	1.39	2.15	1.60	1.10	1.40	1.08	1.89	2.28	1.39	1.34	1.58
Rye	0.82	1.35	2.21			1.44	1.05	1.85	1.70			1.80
L.S.D. (P = .05)	0.22	0.26	N.S.	0.27	0.24		N.S.	0.39	N.S.	0.31	N.S.	
S.E.M. %	7.38	6.43	6.12	5.29	6.51		7.12	7.78	4.96	5.97	7.78	
<i>Grain yields—pounds per acre</i>												
Wheat	281	985	1822	1324	927	1068	360	1042	2096	1412	1493	1281
Oats	489	1700	3250	1602	1742	1757	609	1743	3078	1481	2470	1876
Barley	528	1711	2481	1497	1427	1529	720	2301	2599	1502	1564	1465
Rye	337	883	1859			1026	470	1276	1752			1166
L.S.D. (P = .05)	127	306	725	N.S.	429		270	411	331	N.S.	527	
S.E.M. %	6.03	5.72	8.20	4.51	6.10		4.87	6.11	3.72	4.17	5.65	

yields were obtained when cereal companion crops were used. However, cross-seeding of the companion crop reduced subsequent forage yields less than did companion crops seeded in the drill rows with forage. In the first crop year the mean yields of forage were reduced by 25 to 55 per cent when seeded with companion crops, while at the same time forage yields were reduced by only 25 to 40 per cent when seeded crosswise to the companion crops. In the second crop year the yield depression resulting from the use of companion crops was still evident. However, the decline had shrunk to 20 to 30 per cent for forage seeded with the companion crop, and 10 to 20 per cent for forage seeded crosswise to the cereal companion crop. The range in percentage yield reduction of the forage crop was primarily the result of the method of harvesting the cereal companion crop. Thus, the higher figure in each of the preceding ranges indicates the amount of yield reduction where the cereal companion crops had been mowed for hay while the smaller yield reductions were from plots harvested for grain.

Wheat was the companion crop which generally resulted in the least reduction of first-year forage yield. However, in the second year the reduction in forage yield due to all cereal companion crops was about equal.

In Test 2, where companion crops were seeded crosswise to the forage crop, the cereals were seeded in 6- and 12-inch row spacings. The mean subsequent forage yields as influenced by companion crop row spacings are shown in Table 4. In both first and second crop years the 6-inch row spaced companion crop treatments caused a greater forage yield depression than did 12-inch row spaced companion crops. The average reduction for 6-inch row spacing treatments was 45 per cent in the first crop year and 27 per cent in the second crop year. For the 12-inch row spacing treatments the average yield reductions were 30 per cent in the first year and 18 per cent in the second crop year.

#### *Cereal Companion Crop Yields*

Hay and grain yields obtained from the cereal companion crops in the establishment year are summarized in Table 5. Both hay and grain yields were only slightly lower when the cereals were seeded with the forage crops than when seeded crosswise to forage rows. Oats gave the highest yields of hay or grain while yields of wheat were usually the lowest.

Table 6 shows the mean yield of hay and grain obtained from the cereal companion crops when they were cross-seeded in 6- or 12-inch spaced rows. The yields from both spacings as recorded in the two tests were very similar.

#### DISCUSSION

A comparison of the various measurements occurring in the two tests indicates the consistently smaller forage stand and yield values obtained in Test 1 where the cereals and forage crop were seeded together. This is not surprising when it is considered that maximum competition for moisture, nutrients, and light was forced upon plants of both crops when they were seeded simultaneously in one row at one seeding depth. On the other hand, the cross-seeding technique minimized the competition as much as possible.

TABLE 6.—CEREAL COMPANION CROP HAY AND GRAIN YIELDS FROM 6- AND 12-INCH ROW SPACINGS (TEST 2, CROSS SEEDING)

Cereal companion crop	6-inch row spacings						12-inch row spacings					
	1950	1951	1952	1953	1954	5-year av.	1950	1951	1952	1953	1954	5-year av.
	<i>D.M. hay yields—tons per acre</i>											
Wheat	1.10	1.15	1.93	1.37	0.87	1.28	0.92	1.01	1.86	1.41	0.92	1.22
Oats	1.31	1.84	2.21	2.21	1.11	1.76	1.16	1.61	2.05	1.98	1.27	1.61
Barley	0.93	1.60	2.29	1.43	1.06	1.46	1.08	1.89	2.28	1.39	1.34	1.60
Rye	1.26	1.83	2.01			1.70	1.05	1.85	1.70			1.80
L.S.D. (P = .05)	N.S.	0.38	N.S.	0.33	N.S.		N.S.	0.47	0.32	0.25	N.S.	
S.E.M. %	5.23	8.12	6.22	5.29	7.84		7.12	7.78	4.96	5.97	7.78	
	<i>Grain yields—pounds per acre</i>											
Wheat	572	1034	1988	1467	1522	1317	360	1042	2096	1412	1493	1281
Oats	772	1881	2960	1510	2360	1897	609	1743	3078	1481	2470	1876
Barley	624	2152	2674	1537	1614	1720	720	2301	2599	1502	1564	1537
Rye	588	1210	1810			1203	470	1276	1752			1106
L.S.D. (P = .05)	N.S.	429	485	N.S.	339		261	319	377	N.S.	503	
S.E.M. %	6.35	6.42	4.68	3.49	5.75		4.87	6.11	3.72	4.17	5.66	

Not only were the two crops seeded separately at their particularly adapted seeding depth, but the occurrence of seeds of the two crops at one micro-location was practically eliminated except where the rows intersected.

The method of companion crop harvest had a notable influence on subsequent forage performance. In the first forage crop year the yields following the cereal hay harvest treatment were 20 per cent less than forage yields obtained from plots where the cereal grains were harvested for grain. This difference was still more than 10 per cent in the second forage year and can be primarily attributed to the reduced stand of alfalfa. It was observed that alfalfa seedlings were often killed by the 2-inch clipping treatment received in the hay-mowing process. Consequently, the amount of alfalfa in the stand was reduced and even by the third season the amount of alfalfa in the mowed plots was about two-thirds of that in the plots where companion crops were high-cut for grain (Table 2).

The use of companion crops in narrow row spacings simply added another depressing factor to the already depressed forage yields. In no respect would narrow row spacings for the cereals be warranted, since even the hay or grain yields obtained from 6-inch spaced cereals were no different than those realized from the 12-inch spaced rows.

The choice of the cereal companion crop might seem somewhat debatable. From the standpoint of crop return in the establishment year, oats was undisputably ahead of the other cereals, but if consideration is given to the influence of cereals upon the following forage crop performance, then wheat would be favoured. Since harvesting the companion crop for grain instead of for hay resulted in a stronger, more productive forage crop stand, it seems logical to make wheat the first choice. In the maturing process wheat will shed its leaves more quickly than will oats or barley and thereby permit more light to reach the shorter forage seedlings. The importance of light through companion crops was shown by Flanagan and Washko (3).

Cereal companion crops did reduce the stand, vigour, and early subsequent forage crop yields under dryland prairie conditions at Swift Current. The main advantage which can be claimed for their use was the crop return they offered during the seeding year. These studies have indicated ways and means of management whereby the disadvantages of cereal companion crops in arid regions may be minimized without too seriously reducing the productivity of the perennial forage crop mixture. For the sake of summary the recommended management factors are listed in the following point form:

1. Cross-seed the cereal companion crop.
2. Use wheat as the companion crop.
3. Seed the companion crop at a reduced rate in 12-inch spaced rows.
4. Harvest the cereal as grain at a height of 8 inches or more.

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# COLD HARDINESS OF SPROUTING WHEAT AS AFFECTED BY DURATION OF HARDENING AND HARDENING TEMPERATURE<sup>1</sup>

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## ABSTRACT

The cold hardness of sprouting winter wheat seeds, as measured by exposure to  $-15^{\circ}\text{C}$ . for 16 hours, increased rapidly during the first 5 weeks of hardening and decreased rapidly between the seventh and eleventh week of hardening at  $1.5^{\circ}\text{C}$ . in the dark. With a slightly higher hardening temperature ( $3.5^{\circ}\text{C}$ .) in the dark, a lower level of cold hardness resulted; cold hardness reached a maximum with 4 weeks of hardening and then decreased. Material grown at  $5^{\circ}\text{C}$ . did not develop sufficient cold hardness to withstand the freezing temperature. The application of supplementary light during hardening at  $3.5^{\circ}\text{C}$ . resulted in a slight increase in average hardness but did not prevent the rapid decrease in hardness after the fourth week of growth.

Sprouting winter wheat will harden to cold in the dark. The ultimate level of cold hardness attained depends on the hardening temperature, the duration of hardening, and the stage of development of the seedling. Small changes in these factors can result in large differences in cold hardness.

## INTRODUCTION

Many studies on the nature and practical evaluation of winter hardness in plants have been reported. Extensive reviews of the literature on the subject have been published recently by Dexter (3) and Levitt (6). In general these studies have shown that cold hardness is a major factor concerned with winter hardness. The ultimate level of cold hardness reached in a plant is dependent upon its being grown under conditions conducive to hardening. Hardening to cold may be influenced by temperature, duration of hardening, light, moisture, nutrition, and stage of development of the plant. Optimum conditions for hardening may vary with species and with varieties within a species.

In wheat, most studies of hardening to cold and of relative cold hardness of varieties have been made with plants older than the 1-leaf seedling stage. There are many conflicting reports concerning the relation between age of plant and cold hardness (6) but, in general, it is agreed that hardness or the ability to harden increases between the time the plants emerge from the soil and about the time they reach the 4- to 5-leaf stage. Suneson and Peltier (8) reported that very young seedlings, presumably still dependent upon the endosperm, were much more cold-tolerant than young seedlings that had just emerged from the soil and presumably were on the verge of endosperm independence.

Recently there has been considerable interest in testing for cold resistance of varieties in the coleoptile or sprouting seed stage. Ivanoff (5) was able to distinguish winter hardy strains of oats from spring oats by soaking seeds for 20 hours and then freezing them at an appropriate tempera-

<sup>1</sup>Contribution from the Cereal Breeding Section.



ture. Segeta (7) found high resistance to low temperatures during the period just before the coleoptile and roots rupture the pericarp and a marked decrease as germination progresses. His material was soaked for 18 hours and exposed to various freezing temperatures but no attempt was made to harden it before freezing. Grahl (4) described a method of testing for cold resistance in the coleoptile stage. Seeds were germinated at room temperature until the coleoptiles were 5 mm. long, hardened for 3 days at 0° C. with 16 hours of light per day, then frozen at -5.7° C. After thawing for 1 day at 0° C. they were transplanted to the greenhouse where cold resistance was assessed after 14 days. Dantuma (2) could not obtain consistent results by using the method described by Grahl. Andrews (1) described a method of testing for cold resistance in the sprouting seed stage involving hardening during sprouting in the dark at 0.5° C. for 4 to 6 weeks and exposing to -15° C. for 16 hours. Further experience by the author in the use of this test has confirmed its value but also revealed variation in survival of standard varieties with different durations of hardening and with small differences in hardening and freezing temperatures.

The experiments reported herein were undertaken (1) to investigate the pattern of hardening winter wheat to cold during sprouting; (2) to obtain information relating to the reason for the lack of agreement between results of cold testing at this stage reported in literature, and (3) to determine the reason for the variation of results between tests observed by the author.

#### MATERIALS AND METHODS

The varieties of winter wheat used in this study, Kharkov 22 M.C., Minturki, Nebred, Jones Fife, and Elgin, were chosen because they represent a known range of cold resistance from both field and cold chamber tests. With one exception, which will be described later, all seed was produced at Lethbridge in 1957. All seed was treated with Ceresan diluted with 10 parts talc and hand-picked for freedom from cracks and chips.

The varieties were grown in plastic utility boxes, 3½ x 7 inches, with 10 compartments, as illustrated in a previous publication (1). Ten cc. of fine vermiculite (finish aggregate grade Zonolite) and eight ml. distilled water were added to each compartment. One to two hours later seeds were placed on the surface of this moist substrate. This has been found to be a more satisfactory procedure than that described originally by Andrews (1) because it gives better control of moisture and more uniformity of growth. All material was allowed to germinate for 16 hours at about 22° C. before exposure to the various hardening treatments. Each box was placed in a polyethylene bag to prevent evaporation during the hardening treatments. In all experiments 20 seeds of each variety were used per box compartment and there were 10 replicates of all treatments. A split block design was used in which the varieties were the sub-plots and the treatments the main plots.

Hardening and exposure to freezing temperatures was carried out in walk-in constant temperature chambers 5.5 x 8.5 feet wide with a 7.5-foot ceiling. Temperatures are recorded at one location in each chamber every 3 minutes by means of thermocouples and an automatic recorder. Tempera-

tures in these rooms at the thermocouple seldom varied more than  $\pm 0.5^{\circ}\text{C}$ . During hardening all material was kept in cardboard boxes on shelves at the same level in the hardening room. Thus variation in temperature between treatments within an experiment was at a minimum. The various hardening treatments are described for specific experiments under "Results". Except where otherwise stated, all hardening treatments were carried out in the dark.

To test differential cold hardiness all material was exposed for 16 hours in a chamber controlled at  $-15^{\circ}\text{C}$ . The plastic boxes were placed individually on a wire-mesh rack to ensure uniform exposure to the freezing temperature. After removal from the freezing chamber all material was allowed to thaw for about 5 hours at  $22^{\circ}\text{C}$ . Seedlings were then covered with a sand-vermiculite mixture and moved to a shaded greenhouse maintained at about  $20^{\circ}\text{C}$ . A nutrient solution prepared as described by Andrews (1) was added as necessary.

The number of surviving plants were counted about 2 weeks after exposure to freezing. All percentage data were transformed by means of the angular transformation (angle =  $\arcsin(\text{percentage})$ ) before analysis of variance was applied.

## RESULTS

### *Effect of Duration of Hardening at $1.5^{\circ}\text{C}$ .*

The first experiment involved five varieties, Kharkov 22 M.C., Minturki, Nebred, Jones Fife, and Elgin, and nine hardening treatments. The seed of Kharkov 22 M.C. used in this experiment was, by error, from a stock produced in 1953. All other seed was produced in 1957. There were nine hardening treatments: 1, 2, 3, 4, 5, 6, and 7 weeks at  $1.5^{\circ}\text{C}$ , 1 week at  $5^{\circ}\text{C}$ , and 48 hours at  $22^{\circ}\text{C}$ . The latter two were considered as controls to determine whether hardening was actually taking place at the

TABLE 1.—EFFECT OF DURATION OF HARDENING AT  $1.5^{\circ}\text{C}$ , UPON SURVIVAL OF FIVE VARIETIES OF WINTER WHEAT WHEN EXPOSED TO  $-15^{\circ}\text{C}$ , FOR 16 HOURS

Variety	Mean survival (percentages transformed to angles)							
	Weeks of hardening							Mean
	1	2	3	4	5	6	7	
Minturki	11.0	15.9	26.9	34.2	38.1	43.2	34.3	29.1
Kharkov 22 M.C.	11.1	15.0	26.0	35.2	34.1	35.0	41.8	28.2
Jones Fife	1.3	8.0	6.2	16.0	22.1	25.0	15.2	13.4
Nebred	1.3	0.0	4.4	9.8	17.8	20.5	14.8	9.8
Elgin	0.0	1.3	5.4	14.1	9.5	13.5	13.0	8.1
Mean	4.9	8.0	13.3	21.8	24.3	27.4	23.9	18.0

S.E. of a mean for varieties

1.15

S.E. of a mean for varieties at any one hardening treatment

2.57

S.E. of a mean for hardening treatments

1.26

S.E. of a mean for hardening treatments for any one variety

2.22

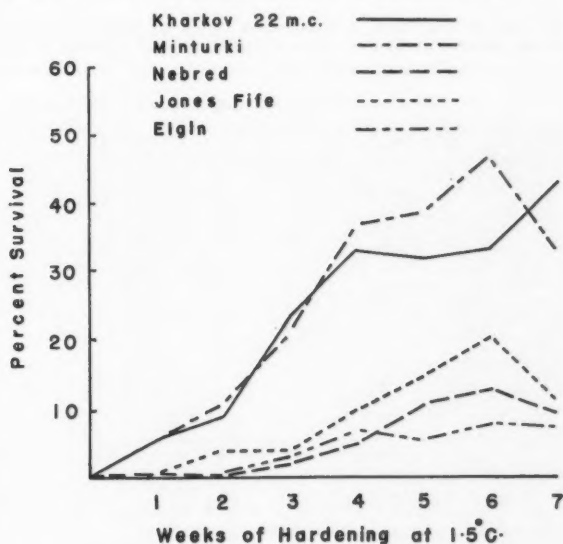


FIGURE 1. Percentage survival of winter wheat varieties hardened for seven periods of 1 to 7 weeks' duration at  $1.5^{\circ}\text{C}$ . and exposed to  $-15^{\circ}\text{C}$ . for 16 hours.

lower temperature. The longest hardening treatment was started first and the shortest last, so that all treatments could be exposed to the freezing temperatures at exactly the same time. This procedure was followed in all experiments.

After exposure to  $-15^{\circ}\text{C}$ . for 16 hours there was no survival in any of the varieties grown for 1 week at  $5^{\circ}\text{C}$ . nor in those grown for 48 hours at  $22^{\circ}\text{C}$ . The percentage survival in the varieties after the seven different periods of hardening at  $1.5^{\circ}\text{C}$ . is shown graphically in Figure 1. The analysis of variance (Table 1) showed a significant difference between means of varieties and treatments ( $P < .01$ ) and a significant variety  $\times$  treatment interaction ( $P < .01$ ). In general there was a progressive increase in hardening during the first 6 weeks at  $1.5^{\circ}\text{C}$ . and, with four of the five varieties, a slight decrease in survival at 7 weeks of hardening.

To obtain additional information on the apparent loss of hardiness after the sixth week of growth at  $1.5^{\circ}\text{C}$ . the experiment was repeated with ten hardening treatments: 2, 3, 4, 5, 6, 7, 8, 9, 10, and 11 weeks' duration at  $1.5^{\circ}\text{C}$ . In this experiment the variety Nebred was replaced by a 1957 stock of Kharkov 22 M.C. Thus two stocks of Kharkov 22 M.C. were included, one produced in 1957 and comparable in age to the other varieties, and one produced in 1953 for comparison with the previous experiment. The stage of development of seedlings in each of the 10 hardening treatments at the time of exposure to freezing is shown in Figure 2.

The percentage survival obtained after exposure to freezing is shown graphically in Figure 3. The varieties Jones Fife and Elgin had very low survival in all treatments and therefore were not included in the analysis

TABLE 2.—EFFECT OF DURATION OF HARDENING AT 1.5° C. UPON SURVIVAL OF THREE VARIETIES OF WINTER WHEAT WHEN EXPOSED TO -15° C. FOR 16 HOURS

Periods of hardening in weeks	Mean survival (percentages transformed to angles)			
	Kharkov 22 M.C.		Minturki	Mean
	1953 seed	1957 seed		
2	25.1	29.6	24.9	26.6
3	31.4	37.9	36.7	35.4
4	28.1	33.9	39.3	33.8
5	40.2	49.7	53.7	47.9
6	48.0	47.2	54.1	49.8
7	41.7	46.7	35.2	40.5
8	28.5	26.6	23.3	26.1
9	18.7	15.2	22.2	18.7
10	14.4	10.7	16.1	13.7
11	11.3	5.7	19.1	12.0
Mean	28.7	30.1	32.5	30.4

S.E. of a mean for varieties

0.88

S.E. of a mean for varieties at any one hardening treatment

2.55

S.E. of a mean for hardening treatments

1.75

S.E. of a mean for hardening treatments for any one variety

2.8

of variance. Their lower level of cold hardiness is obvious. As shown in Table 2, there was a significant difference between variety means and between treatment means ( $P < 0.01$ ).

In this experiment a progressive increase in cold hardiness during the first 5 weeks at 1.5° C. was evident. After the sixth week of growth at 1.5° C. there was a rapid decrease in cold hardiness.

To determine the degree of vernalization that had taken place during the hardening period, 15 surviving seedlings of both Kharkov 22 M.C. (1957 stock) and Minturki from each of the hardening treatments were transplanted into crocks in the greenhouse and grown to heading. The percentage of plants headed at each of four dates after transplanting is given in Table 3. Plants not headed at the last date recorded had still not headed 4 months later, and it seemed quite likely that their failure to head was due to freezing damage, as they were also weak and abnormal in growth habit. This probably was also the reason for the lower percentage of plants headed from the 11-week treatment. The rapid reduction in cold hardiness after the sixth week at 1.5° C. (Figures 1 and 3) coincides with the completion of the vernalization process (Table 3). Minturki appeared to require a shorter vernalization time than Kharkov 22 M.C. However, vernalization studies of the five varieties tested (Figure 1), with material not exposed to freezing, showed that Kharkov 22 M.C., Minturki, and Jones Fife all required about the same vernalization time, 6 weeks (Table 4). Nebred required about 5 weeks of vernalization and Elgin was intermediate. Thus the relative cold hardiness and vernalization requirement of these varieties does not appear to be directly related, although completion of vernalization and the reduction in cold hardiness occurs at about the same stage of development.

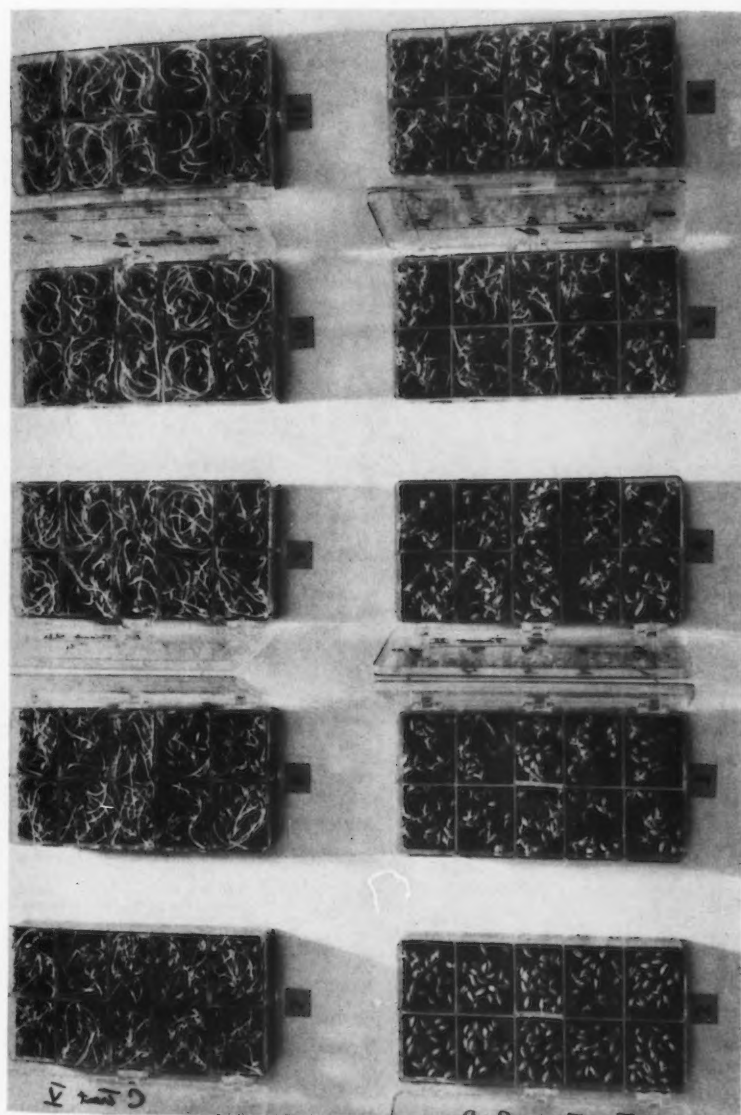


FIGURE 2. Relative stages of development of winter wheat grown for 10 periods of 2 to 11 weeks' duration at 1.5° C.



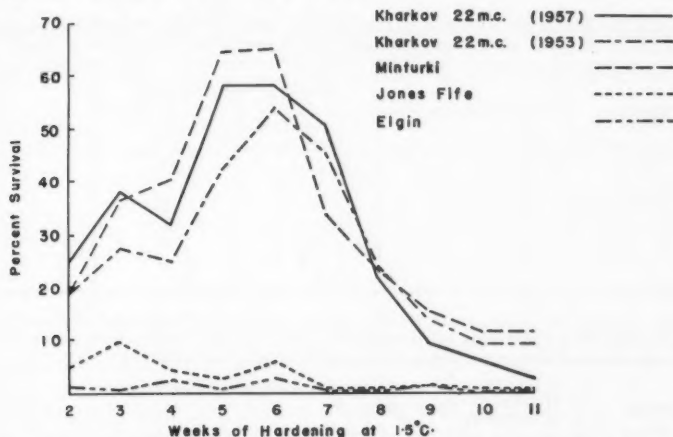


FIGURE 3. Percentage survival of winter wheat varieties hardened for 10 periods of 2 to 11 weeks' duration at 1.5° C. and exposed to -15° C. for 16 hours.

#### *Effect of Temperature on Hardening During Sprouting*

To determine the effect of small differences in temperature on hardening during sprouting, Kharkov 22 M.C. wheat was hardened for 1, 2, 3, 4, and 5 weeks' duration at each of three temperatures: 1.5°, 3.5°, and 5° C. Coleoptile lengths at the end of each treatment are compared in Table 5. All treatments were frozen simultaneously at -15° C. for 16 hours. The relative survival for the various hardening treatments are given in Table 6. There was no survival for any material grown at 5° C. At 3.5° C. maximum survival was obtained in the 4-week treatment and survival was lower in the 5- than in the 4-week treatment, while at 1.5° C. at least 5 weeks of hardening were required for maximum survival and a higher percentage survival

TABLE 3.—PERCENTAGE OF SURVIVING PLANTS OF KHARKOV 22 M.C. AND MINTURKI WINTER WHEAT THAT HEADED AFTER BEING HARDENED AT 1.5° C. FOR SPECIFIC PERIODS AND EXPOSED TO -15° C. FOR 16 HOURS

Weeks of hardening	Kharkov 22 M.C.				Minturki			
	Days after transplanting				Days after transplanting			
	105	111	118	125	105	111	118	125
2	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	13	28
6	0	0	0	0	0	13	33	40
7	0	0	0	23	20	27	33	40
8	15	47	80	86	42	58	76	84
9	40	71	71	71	33	47	60	66
10	61	76	76	100	60	73	80	80
11	23	23	46	62	53	66	60	66



TABLE 4.—RELATIVE HEADING DATES IN AUGUST OF WINTER WHEAT VARIETIES VERNALIZED FOR VARIOUS DURATIONS AND PLANTED IN THE FIELD IN JUNE

Variety	Weeks of vernalization			
	4	5	6	7
Minturki	—	—	12	7
Kharkov 22 M.C.	—	—	13	9
Jones Fife	—	—	16	5
Elgin	—	27	7	5
Nebred	—	9	5	5

TABLE 5.—EFFECT OF DURATION OF HARDENING AND OF HARDENING TEMPERATURE UPON COLEOPTILE LENGTHS OF KARKOV 22 M.C. AND MINTURKI WINTER WHEAT

Variety	Hardening temperature	Weeks of hardening					
		1	2	3	4	5	6
		mm.	mm.	mm.	mm.	mm.	mm.
Kharkov 22 M.C.	1.5° C.	1	3	8	14	17	—
	3.5° C.	2	5	12	17	30	—
	5° C.	4	15	45	63	88	—
Minturki	1.5° C.	1	2	5	9	15	24
	3.5° C.	2	4	8	20	40	58

TABLE 6.—EFFECT OF DURATION OF HARDENING AND OF HARDENING TEMPERATURE UPON SURVIVAL OF KHARKOV 22 M.C. AND MINTURKI WINTER WHEAT WHEN EXPOSED TO -15° C. FOR 16 HOURS

Variety	Hardening temperature	Weeks of hardening					
		1	2	3	4	5	6
		%	%	%	%	%	%
Kharkov 22 M.C.	1.5° C.	1.5	4.5	7.5	31.0	35.0	—
	3.5° C.	4.0	0.5	2.0	16.5	2.5	—
	5° C.	0	0	0	0	0	—
Minturki	1.5° C.	20.5	21.0	32.5	37.5	44.0	36.5
	3.5° C.	12.5	5.0	4.5	25.0	1.5	2.0

was obtained. Differences between means for hardening temperatures and for weeks of hardening at each temperature were significant ( $P < .01$ ).

A similar experiment was carried out at a later date involving Minturki wheat hardened for 1, 2, 3, 4, 5, and 6 weeks' duration each at 1.5° and 3.5° C. All material was exposed simultaneously to -15° C. for 16 hours. Coleoptile lengths for the various treatments are compared in Table 5 and survival data in Table 6. There was a significant difference ( $P < .01$ ) between means for hardening temperatures and for weeks of hardening at each temperature. As with Kharkov 22 M.C., maximum survival was obtained with 4 weeks of hardening at 3.5° C. and with 5 weeks of hardening at 1.5° C. Survival was higher at the lower hardening temperature.

TABLE 7.—EFFECT OF DURATION OF HARDENING AND OF HARDENING TEMPERATURES WITH AND WITHOUT LIGHT UPON SURVIVAL OF KHARKOV 22 M.C. WINTER WHEAT WHEN EXPOSED TO  $-15^{\circ}\text{C}$ . FOR 16 HOURS

Hardening temperature	Hours of light per day	Weeks of hardening					
		1	2	3	4	5	6
		%	%	%	%	%	%
$3.5^{\circ}\text{C}$ .	0	0.5	4.0	12.0	19.0	5.5	0.0
$3.5-4.5^{\circ}\text{C}$ .	8	3.5	8.0	14.0	24.5	8.5	1.0
$6^{\circ}\text{C}$ .	24	0	0	0	0	0	0

It is obvious that a greater degree of hardening takes place at the lower temperatures and that hardening or the ability to harden is related to stage of growth. At the higher hardening temperature ( $3.5^{\circ}\text{C}$ .), where growth is more rapid, maximum hardening was reached in a shorter period of time and, also, retained for a shorter period. At  $3.5^{\circ}\text{C}$ ., with both Kharkov 22 M.C. and Minturki (Table 6), survival was higher at 1 week than at 2 or 3 weeks of hardening. This was not evident at  $1.5^{\circ}\text{C}$ . nor in results given in Table 7.

#### *Effect of Light on Hardening and Cold Hardiness*

Studies concerning light were carried on only to determine whether or not the reduction in cold hardiness found after a certain stage of development in the dark could be influenced by light. Satisfactory equipment was not available for more detailed studies of the effect of light.

Kharkov 22 M.C. winter wheat was grown in a constant-temperature room controlled at  $3.5^{\circ}\text{C}$ . and subjected to the following hardening treatments:—

- (1) Periods of 1, 2, 3, 4, 5, and 6 weeks in the dark.
- (2) Periods of 1, 2, 3, 4, 5, and 6 weeks with 8 hours of light per day provided by four 40-watt standard daylight fluorescent tubes and four 60-watt incandescent bulbs with a glass sheet separating lights and seedlings.
- (3) Periods of 1, 2, 3, 4, 5, and 6 weeks with 24 hours of light per day provided by ten 40-watt fluorescent tubes and eight 25-watt incandescent bulbs with no glass separation between lights and seedlings.

Light banks were about 18 inches above the growing material.

At the level of the sprouting seeds the temperature for the 8-hour light treatment was  $3.5^{\circ}\text{C}$ . with lights off and  $4.5^{\circ}\text{C}$ . with lights on and for the 24-hour light treatment  $6^{\circ}\text{C}$ .

The percentage survival in the different hardening treatments after simultaneous exposure to  $-15^{\circ}\text{C}$ . for 16 hours is given in Table 7. Coleoptile lengths of seedlings grown in the dark were respectively, 1, 3, 5, 12, 45, and 55 mm. after each of the 1 to 6 weeks of growth, but the leaves had not broken through the coleoptiles at the sixth week. With 8 hours of light, coleoptile lengths were comparable to the dark treatment up to the third week, but at that stage leaves had started to break through the coleoptiles. Chlorophyll was evident in all treatments involving light. With continuous light, plants were in the 1-leaf stage at 2 weeks and in the 3-leaf stage at 6 weeks of growth.

None of the material grown under continuous light at 6° C. hardened sufficiently to survive the freezing temperature. The mean survival with 8 hours of light per day at 3.5° C. was significantly higher ( $P < .05$ ) than that with the dark treatment at 3.5° C. However, the pattern of increasing hardness during the first 4 weeks of growth and decreasing hardness thereafter was similar with or without light (Table 7).

#### DISCUSSION

It is evident that winter wheat will harden to cold during sprouting. The level of cold hardness attained is dependent upon the hardening temperature, the duration of hardening, and the physiologic age of the seedlings, the influence of the latter two being difficult to separate. During sprouting hardening is apparently dependent upon conversion of stored food reserves in the endosperm, and although light may affect this hardening, it is not essential. During sprouting at 1.5° or 3.5° C. maximum hardening is reached when coleoptiles are between about 15 and 30 mm. in length.

In two experiments at 3.5° C. there was higher survival after 1 week than after 2 weeks of hardening. This agrees with Segeta's (7) report of high resistance to low temperature during the period just before the coleoptile and roots rupture the pericarp and with his report of a marked decrease as germination progressed.

It appears that, under appropriate conditions of hardening, cold hardness of wheat may reach a maximum at three physiologic ages of growth. The first occurs when coleoptiles and roots are less than 1 mm. in length, and it is followed by a decrease as growth progresses (7). The second occurs when coleoptiles have reached a length of about 15 mm., and this also decreases when coleoptiles are about 30 mm. in length. The length may vary somewhat with variety and differences in hardening temperature. The decrease in cold hardness at this stage is probably associated with the exhaustion of certain food reserves in the endosperm, although growth will continue for a considerable period beyond the 30-mm. coleoptile length with no nutrients supplied. A third maximum occurs at about the 5-leaf stage, and this also is followed by a decrease (6, 9). Hardening at this stage is dependent upon reserves produced by photosynthesis.

In testing for cold hardness in the coleoptile stage it is evident that small differences in hardening temperature and period of hardening can greatly influence the results obtained. At 1.5° C. best differentiation between varieties was obtained after 5 or 6 weeks of hardening. At an older or younger stage varietal differences may be reduced greatly. Slight increases in hardening temperatures not only may reduce the degree of hardening obtained but will reduce the time at which a maximum is reached. Thus it is not surprising that widely varying results of attempts to test for cold hardness at this stage have been reported. Best differentiation of varieties may be obtained only with specific conditions of hardening and exposure to freezing.

It was found that the rapid reduction in cold hardness after the sixth week of growth at 1.5° C. was associated with completion of the vernalization process. A parallel between length of vernalization period required

and cold hardiness of varieties has been reported by numerous workers (6). This does not mean that the two are governed necessarily by the same or closely related processes because many varieties with low winter hardiness also require a long vernalization process. However, the relationship is important when it is desired to grow to maturity the survivors from cold hardiness tests of sprouting wheat. If exposure to freezing is delayed until vernalization is complete, there is considerable danger that the level of cold hardiness and differences between varieties in cold hardiness will be greatly reduced. Theoretically it would seem better to expose to freezing with 5 or 6 weeks of hardening at 1.5° C., when hardening is at a maximum but vernalization is not complete, and then return the material to the hardening room for 1 or 2 weeks to complete vernalization before transplanting.

Winter wheat hardened in a manner similar to that reported herein should offer excellent material for biochemical or other analysis of changes occurring during hardening. Such seedlings can be grown in a shorter period of time, with better control of environmental factors during hardening, and in a smaller space than older plants.

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# TWO VIRUS DISEASES OF RHUBARB IN EASTERN ONTARIO<sup>1</sup>

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## ABSTRACT

Two viruses were isolated from Ruby Red rhubarb growing in the Ottawa district. Isolate I produced a severe rugosity and mottle on rhubarb, a systemic mottle in *Nicotiana tabacum*, *N. glutinosa* and *Datura stramonium*, and local lesions on *Phaseolus vulgaris*, *Vigna sinensis* and *Beta vulgaris*. The virus was inactivated after being heated for 10 minutes at 65° C., and became non-infective after the sap was diluted to 1:2000. Isolate II incited the formation of chlorotic and necrotic spots on rhubarb, and produced a systemic mottle in *N. tabacum*, *N. glutinosa*, and *D. stramonium*. The virus was inactivated by being heated at 75° C. for ten minutes, and became non-infective after the sap was diluted 1:8000. Both isolates were mechanically transmitted by diluting crude sap 1:100 in 0.1M. phosphate buffer (pH 8-9) and rubbing on leaves dusted with carborundum. The success in isolating these viruses or virus strains was dependent on the season in which isolations were attempted. The size of the virus particles of both isolates was 478 x 15  $\mu$ m.

## INTRODUCTION

Rhubarb, *Rheum officinale* L., is an important garden crop in Canada. In spite of cultural practices which favour the acquisition and spread of viruses, there are relatively few references to virus diseases of this crop.

In 1924, Dickson (3) described a mosaic disease of rhubarb in the Montreal district of Quebec. The symptoms consisted of mottling, dwarfing and chlorosis. All attempts to transmit this virus were unsuccessful. Chamberlain (2) in 1946 described a mosaic disease of rhubarb in New Zealand. From his description, this mosaic was similar to that described by Dickson. Chamberlain, however, succeeded in transmitting the virus from rhubarb to broad-leaved dock (*Rumex obtusifolius*) although he was unable to transmit back to rhubarb. The virus was transmitted from dock to dock by the aphids *Myzus persicae* and *Macrosiphum euphorbiae*.

Vaughn (8) in 1953 reported a "ringspot-like virus" of rhubarb which was transmitted, by rubbing, to a variety of hosts. He suggested that the symptoms of this virus on different hosts resembled those of tomato ringspot virus isolated from cucumber. Yale and Vaughn (9) in 1954 succeeded in transmitting a virus from rhubarb to cucumber, bean, Sweet William and zinnia. They also reported that symptoms in rhubarb were masked at temperatures above 65° F. A virus from rhubarb was transmitted to cucumber by Klinkowski (6). He noted a variation of symptoms at different seasons, and observed a bluish fluorescence in the shoots, petioles, and leaf veins of diseased plants.

Examination of rhubarb plantings on the Central Experimental Farm in Ottawa, Ontario, and in the Ottawa district, revealed a variety of symptoms which might be attributed to virus infection. This paper describes two of the viruses isolated from infected plants collected in the Ottawa district.

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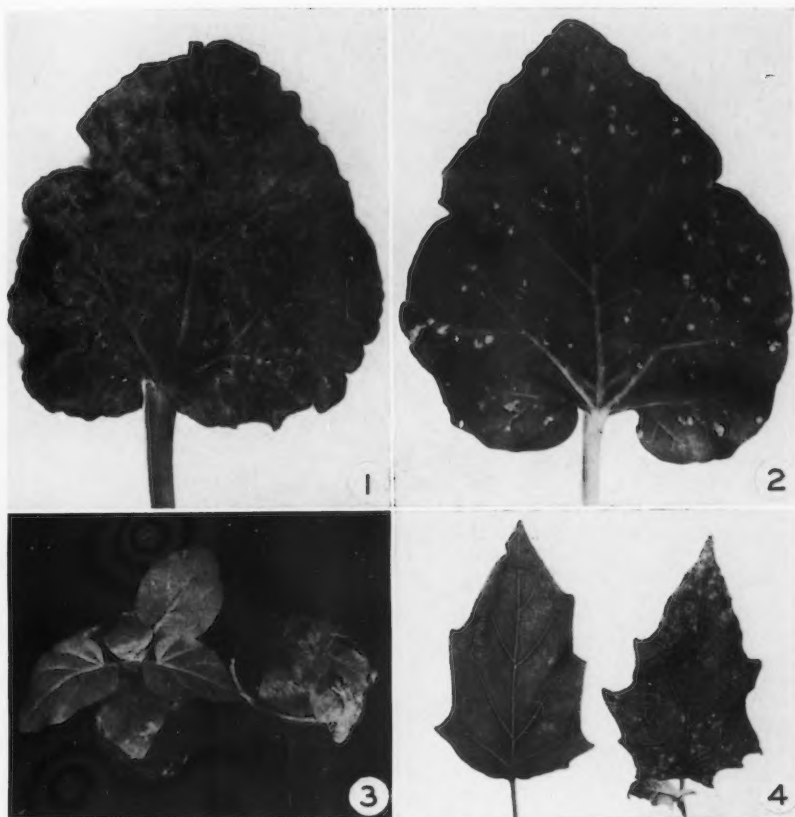


FIGURE 1. Rhubarb leaf showing mottle and rugosity due to infection with Rhubarb Virus I.

FIGURE 2. Rhubarb leaf showing chlorotic and necrotic spots due to infection with Rhubarb Virus II.

FIGURE 3. *N. glutinosa* plants: plant on right infected with virus from rhubarb.

FIGURE 4. Leaves of *D. stramonium*: leaf on right shows mottle following infection with virus from rhubarb.

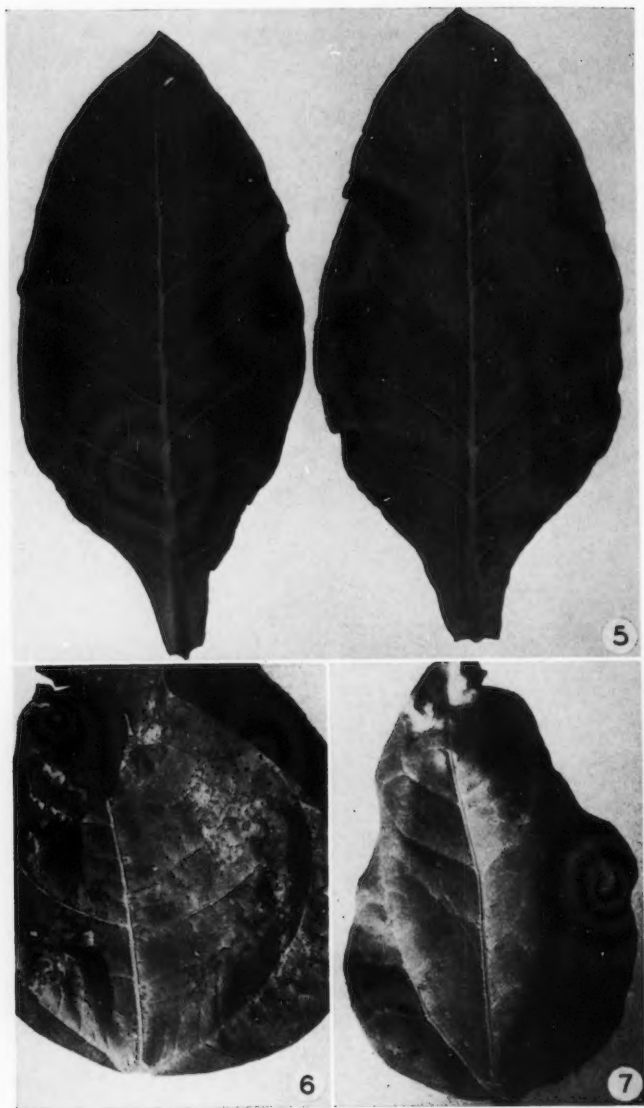


FIGURE 5. Leaves of *N. tabacum*: leaf on right shows fleeting mottle following infection with rhubarb virus.

FIGURE 6. Local lesions on leaf of *P. vulgaris* following inoculation with Rhubarb Virus I.

FIGURE 7. Local lesion on leaf of *V. sinensis* following inoculation with Rhubarb Virus I.



### Materials and Methods

In January, 1957, 15 roots of the Ruby Red variety of rhubarb were collected at random from the field and planted in 10-inch pots in the greenhouse. The plants were maintained at a temperature of approximately 75° F., and the day length was extended by using artificial lighting from 5 to 12 p.m. daily. To prepare the inoculum, the crude sap from leaves and petioles was diluted 1:100 with 0.1M phosphate buffer (pH 7.0). All inoculations were made by rubbing with a glass spatula on plants which had been previously dusted with 500-mesh carborundum. After inoculation, the indicator hosts were held at 75° F. and received supplementary light from 5 to 12 p.m. daily.

### EXPERIMENTAL RESULTS

#### *Virus Symptoms Rhubarb*

Of the 15 plants grown in the greenhouse, 13 showed symptoms that indicated virus infection. Five of these plants showed a severe rugosity and mottle (Figure 1), and on the leaves of the remainder there were distinct, circular, chlorotic spots which became necrotic as the leaves matured (Figure 2).

#### *Differential Hosts*

Sap from the infected rhubarb plants was rubbed on five of each of the following plants: *Nicotiana tabacum* L., var. Havana 38; *Nicotiana glutinosa* L.; *Datura stramonium* L.; *Lycopersicon esculentum* Mill., var. Stokesdale; *Gomphrena globosa* L.; *Chenopodium amaranticolor* Coste & Reyn.; *Petunia hybrida* Vilm.; var. Fire Chief; *Cucumis sativus* L. var., Straight Eight; *Phaseolus vulgaris* L. vars. Great Northern, Pencil Pod, Longgreen, Perry Marrow and Red Kidney; *Callistephus chinensis* Nees.; *Zinnia elegans* Jacq.; *Vigna sinensis* Endl.; *Physalis floridana* Rydb.; *Beta vulgaris* L.; *Nicandra physaloides* (L.) Pers. and *Gomphrena globosa* L.

Some plants of *N. glutinosa* and *D. stramonium* developed a severe mottle 14 to 20 days after inoculation (Figures 3 and 4), while some of the inoculated plants of *N. tabacum* developed a fleeting mottle in 25 to 30 days (Figure 5). When the inoculum was taken from rhubarb with chlorotic leaf spots, symptoms developed only on *N. glutinosa*, *N. tabacum* and *D. stramonium*, and in most instances, only one or two of the five inoculated plants showed symptoms. When inoculum was taken from plants showing severe rugosity and mottle, local lesions were observed on *P. vulgaris* (Figure 6) and *Vigna sinensis* (Figure 7) 3 days after inoculation. Necrotic rings and half rings developed on *Beta vulgaris* 3 weeks after inoculation. All plants inoculated with sap from rhubarb showed severe injury, even when they were rubbed very lightly.

When the inoculum was taken from infected plants of *N. glutinosa*, *N. tabacum*, or *D. stramonium*, and rubbed on healthy plants of these species, symptoms developed in from 5 to 7 days, and no injury was apparent.

On the basis of symptoms on rhubarb, and the differential host reaction, it appeared that only two viruses or two strains of a single virus were present in the 13 infected rhubarb plants.

Transmission of the viruses from *N. glutinosa* to rhubarb seedlings of the varieties Ruby Red, Macdonald, Valentine and Linnaeus was attempted. No symptoms were observed in any of the seedlings during the season of inoculation. After the seedlings had been placed in cold storage for 3 months and brought into the greenhouse again, it was found that 82 per cent of the Ruby Red plants showed symptoms identical to those on the plants from which the virus was originally isolated. Eighteen per cent of the Macdonald plants were infected, but plants of the Linnaeus and Valentine varieties were apparently healthy. The presence of virus in the Ruby Red and Macdonald plants was determined by inoculation to the indicator hosts. In the horticulture plots at Ottawa, no virus symptoms were observed in, and no viruses were isolated from, Linnaeus or Valentine plants, but a high percentage of Ruby Red and Macdonald plants were infected.

#### *Rhubarb Virus Nomenclature*

To date, in the nomenclature of rhubarb viruses, the diseases have been called mosaics, or "ring-spot-like viruses". Undoubtedly, as research on rhubarb viruses continues, it will be found that several different viruses will produce mosaics, spots, or ringspots. Klinkowski (7) has proposed that the virus he has isolated from Rhubarb in Germany be designated as Rheum Virus I or *Marmor rhei*. This method of nomenclature, if adopted now, would aid in preventing confusion in the nomenclature of rhubarb viruses. We had originally decided to name the two isolates described in this paper "Rhubarb Virus I" and "Rhubarb Virus II". We are now attempting to determine the relationship of our virus isolates to that of Klinkowski by serological and other methods. On the basis of these results, it is proposed to name the isolates according to the system initiated by Klinkowski.

#### *Physical Properties*

Rhubarb Virus I was infective after being heated for 10 minutes at 60° C. but was inactivated at 65° C. The virus became non-infective after the sap was diluted in excess of 1:2000.

Rhubarb Virus II was infective after being heated for 10 minutes at 70° C. but was inactivated at 75° C. No infection was obtained with dilutions in excess of 1:8000.

#### *Election Microscopy*

Samples of virus for election microscopy were obtained by subjecting infected plants of *D. stramonium* and *N. glutinosa* to the water pressure procedure as described by Johnston (5). Droplets of sap were collected with a hypodermic needle. When 0.5 ml. was collected, the sap was centrifuged for 30 minutes at 10,000 r.p.m. Grids were then prepared from the supernatant.

The virus particles from different hosts were of comparable size. There was also no detectable difference in size of the two virus isolates. The average size was 478 x 15  $\mu$ m.

#### *A Virus Inhibitor in Rhubarb*

Although the original virus isolates were obtained from rhubarb by dilution of the crude sap by 1:100 in 0.1M phosphate buffer (pH 7.0) and

rubbing on indicator hosts, later attempts to recover the virus by this method were unsuccessful. These results indicated that an inhibitor was present in rhubarb, that the virus concentration had dropped to a level where it could not be recovered, or that the virus in the plant had been inactivated. The continued appearance of symptoms in the plants suggested that the virus was still present.

In an attempt to discover if an inhibitor was present, the effect of crude rhubarb sap on the infectivity of potato viruses X and Y, and on a strain of tobacco necrosis virus was determined. Each of the above-mentioned viruses was diluted 1:10 with crude rhubarb sap, and also with a 1:100 dilution of crude rhubarb sap. Five minutes after dilution, the virus preparations were rubbed on the indicator hosts. The hosts were *Datura stramonium* for Potato X, *Nicotiana glutinosa* for Potato Y, and *Phaseolus vulgaris* var. Red Kidney for the tobacco necrosis strain.

The crude sap from healthy rhubarb plants completely inactivated the Potato X and Potato Y viruses, and delayed the appearance of lesions caused by tobacco necrosis virus infection in bean by 5 to 6 days. The 1:100 dilution of rhubarb sap delayed the appearance of symptoms with all three viruses.

These results indicated that the inhibitor was probably present in rhubarb in low concentration, and that it lost its inactivating effect after being diluted by more than 1:100.

#### *The Effect of Acidity of Inoculum on Virus Isolation*

In early June, 1959, young leaves of the varieties Macdonald, Valentine, Ruby Red, Crimson Queen, Rubet, and Vineland which showed a well defined mottle were collected in the field. The leaves of each variety were ground separately in a meat grinder, and the juice extracted by squeezing through four layers of cheesecloth. The pH of the crude sap was taken, and then one ml. aliquots of the sap of each variety were mixed with 10 ml. of buffer of pH 6.0, 7.0, 8.0, 9.0, 10.0, and 11.0. The pH of the resulting mixtures was taken and the readings are recorded in Table 1.

TABLE 1.—THE EFFECT OF DILUTING RHUBARB SAP WITH BUFFER ON THE pH OF THE MIXTURE

Rhubarb variety	Dilution	Crude sap pH	pH of Buffer					
			6.0	7.0	8.0	9.0	10.0	11.0
			pH of buffer—sap mixture					
Macdonald	1:10	3.4	4.6	6.7	7.3	7.4	7.5	10.0
Valentine	1:10	3.6	4.6	6.7	7.3	7.4	7.5	8.9
Ruby Red	1:10	3.8	5.2	6.9	7.4	7.7	7.8	10.4
Crimson Queen	1:10	3.6	4.9	6.8	7.3	7.6	7.6	9.7
Rubet	1:10	3.3	4.4	6.6	7.1	7.3	7.4	8.6
Vineland	1:10	3.2	4.2	6.6	7.1	7.2	7.3	8.7

The crude sap from each variety and the eight buffer dilutions was rubbed on a series of three plants each of *N. glutinosa*, *N. tabacum* and *D. stramonium*.

The pH of the crude sap varied from 3.2 for Vineland to 3.8 for Ruby Red. No infection of the indicator hosts occurred when undiluted crude sap was rubbed on them. No infection was obtained with preparations below pH 6.6, and the highest percentage of plants were infected in the range pH 6.6 to 7.4.

*The Effect of Season on the Isolation of Virus from Rhubarb*

Although an inhibitor had been demonstrated to be present in rhubarb, repeated attempts through October and November, 1958, to isolate viruses from plants which were known to be infected, by diluting out the inhibitor, failed. In the last week of December, it was again possible to isolate virus from some rhubarb plants by diluting the sap from 1:10 to 1:300. By mid-January no difficulties were encountered in recovering virus from any of the infected plants. From the results obtained in 1957 and 1958, it appeared that the success in isolating virus from rhubarb was quite dependent on the time of year in which the isolations were attempted.

#### DISCUSSION AND CONCLUSIONS

Although it was possible to transmit virus from rhubarb to other hosts, only a small percentage of these hosts became infected. In some cases, transmission of virus from rhubarb plants which were known to be infected could not be accomplished unless large numbers of inoculations were attempted. In that the rhubarb virus isolates could be readily transmitted between hosts, other than rhubarb, it was apparent that some factor or factors in the rhubarb plant were interfering with transmission.

Allsopp (1) and Forest (4) have shown that the juice extracted from rhubarb was very acid, and that this acidity varied considerably with the growing season, the maturity of the plant, and the amount of shading. In the experiments reported in this paper, it was possible to facilitate the transfer of virus from rhubarb by adjusting the pH of the sap to pH 7.0 to 8.0. There is no doubt that some other factor or factors are present in rhubarb which have a marked effect on the virus. Experiments are now in progress to try to determine the nature of these inhibiting materials.

In this paper, the viruses inciting the two distinct systems in rhubarb are designated only as virus isolates. Serological work is now in progress to determine the relationship of these isolates to each other, as well as to the Rheum Virus I described by Klinkowski (7).

Virus-infected rhubarb plants showed a marked loss of vigour, and infected plants in the field appeared to be more susceptible to frost injury. This may account for some of the damage to rhubarb plantings which has previously been unexplained.

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**LA TRANSMISSION PAR LES INSECTES DE  
*CORYNBACTERIUM SEPEDONICUM*  
(SPIECK. & KOTT.) SKAPTASON ET BURKHOLDER**

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**RÉSUMÉ**

Des expériences faites sous cages, en serre, indiquent que des insectes du feuillage de la pomme de terre tels que le doryphore, la cicadelle, l'aphrophore, la punaise terne et le puceron peuvent transmettre l'agent pathogène de la flétrissure bactérienne. L'infection a été obtenue par l'inoculation de plantes saines, soit avec des suspensions d'insectes broyés ayant séjourné sur des plantes malades, soit avec des suspensions de feuilles ou de plantes lyophilisées ayant hébergé des insectes contaminés. L'infection a aussi été effectuée sous cage par le libre mouvement des insectes d'une plante malade à une plante saine.

A l'exception de l'altise, tous ces insectes ont pu infecter, en serre, des pieds de pommes de terre qui produisirent un certain nombre de tubercules infectés mais ne manifestant aucun symptôme. Le doryphore a été le seul insecte capable d'infecter des plantes dont les tubercules-filles ont parfois donné naissance à des tiges manifestant des symptômes de la flétrissure.

**INTRODUCTION**

Il est reconnu, depuis plusieurs années, que la dissémination de la flétrissure bactérienne de la pomme de terre, *Solanum tuberosum* L., causée par *Corynebacterium sepedonicum* (Spieck. et Kott.) Skaptason et Burkholder, s'effectue principalement à la plantation ou à la récolte. Les principaux agents de dissémination sont une semence contaminée, les outils, les instruments aratoires, les contenants, les caves, les entrepôts, etc.

Une fois que toutes les précautions ont été prises en vue d'enrayer la maladie, il est parfois difficile d'expliquer son apparition soudaine dans une plantation à moins qu'il y ait eu transmission au cours de la période de végétation.

Depuis que cette maladie a été signalée au Canada par Baribeau (1) en 1931 et aux États-Unis par Bonde (2) en 1932, peu de chercheurs ont étudié le rôle que pourraient jouer les insectes dans la transmission de l'agent pathogène de la flétrissure bactérienne. Brentzel et Munro (3) n'ont pas réussi à causer d'infection à l'aide des criquets. List et Kreutzer (4) ont observé dans leurs travaux, en serre, que le doryphore infecta trois plantes et que la cantharide en infecta une autre. Ces résultats ne constituent pas d'après eux une preuve tangible de la transmission dans une plantation de l'agent pathogène par les insectes, mais ils justifient la poursuite de recherches plus approfondies.

Voilà pourquoi on entreprit des études sur la possibilité de la transmission de la flétrissure bactérienne par les insectes du feuillage de la pomme de terre.

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### MATÉRIEL

Les insectes à l'étude sont ceux que l'on observe le plus communément sur le feuillage de la pomme de terre, à savoir: l'altise, *Epitrix cucumeris* (Harr.), le doryphore *Leptinotarsa decemlineata* (Say), les cicadelles, en particulier la cicadelle de l'Aster, *Macrosteles fascifrons* (Stal), la punaise terne, *Lygus lineolaris* (P. de B.), les pucerons, en particulier le puceron du pêcher, *Myzus persicae* (Sulz.) et plusieurs espèces d'aphrophores de la famille des cercopides qui envahissent les plantations vers la mi-été.

Le doryphore, les pucerons et les altises proviennent de champs de pommes de terre tandis que les cicadelles, les punaises ternes et les aphrophores ont été collectionnés dans les potagers et sur les mauvaises herbes.

Les insectes recueillis sont introduits par espèce, dans des cages d'élevage en serre où croissent des plantes partiellement flétries, inoculées avec une culture pure de *C. sepedonicum*. Les cicadelles sont cependant déposées dans des cages où croissent des tiges d'orge ou de blé avant leur transfert sur des plantes flétries de la variété Montagne Verte.

### MÉTHODES ET RÉSULTATS

#### Méthode A

Après un séjour de trois à quatre jours au contact des plantes malades, les insectes sont triturés dans un mortier de verre préalablement nettoyé à l'alcool. La trituration de chaque espèce d'insectes, diluée dans quelques gouttes d'eau distillée, sert à inoculer les racines de jeunes plants sains provenant de tubercules de catégorie Fondation. Ces racines sont coupées à leur extrémité avant d'être trempées durant une minute dans chaque suspension d'inoculum. Un ou deux germes prélevés au talon de chaque tubercule tiennent lieu de témoin.

Après leur passage sur des plantes flétries, un certain nombre de doryphores sont désinfectés dans une solution d'hypochlorite de calcium à une concentration de 5 p. 100 durant au moins une minute. Ces insectes sont ensuite lavés à l'eau distillée avant d'être triturés.

Le diagnostic de la maladie sur les plantes inoculées avec les insectes triturés a été basé sur la manifestation des symptômes extérieurs aussi bien que sur l'examen microscopique des empreintes prises à la base de chaque tige. Cet examen microscopique a été fait 100 jours après l'inoculation. Les plantes témoins étaient examinées de la même façon.

#### Résultats

L'examen du tableau 1 indique que le doryphore, la punaise terne, la cicadelle et l'aphrophore se sont contaminés lors d'un séjour de quatre jours sur des plantes atteintes de flétrissure bactérienne. En effet, la plupart des plantes inoculées avec la trituration de ces insectes révélaient la présence de bactéries à l'examen microscopique. Toutefois, le flétrissement caractéristique ne se manifesta que sur les plantes inoculées avec le doryphore, la cicadelle et l'aphrophore. Par contre, l'altise triturée n'a pas causé d'infection.



TABLEAU 1.—ÉTAT SANITAIRE DES PLANTES INOCULÉES PAR LES RACINES AVEC UNE SUSPENSION DE TRITURATION D'INSECTES AYANT VÉCU 4 JOURS SUR DES PLANTES FLÉTRIÉS

Espèce d'insecte	Plantes inoculées	Plantes flétries	Plantes infectées non flétries	Plantes saines
Doryphore <sup>1</sup>	6	4	2	0
Doryphore <sup>2</sup>	8	4	2	2
Altise	5	0	0	5
Punaise terne	5	0	5	0
Cicadelle	9	2	6	1
Aphrophore	5	3	2	0

<sup>1</sup> Insectes désinfectés avec hypochlorite de calcium avant trituration<sup>2</sup> Insectes non désinfectés avec hypochlorite de calcium avant trituration

Parmi ces insectes, le doryphore semble le vecteur le plus efficace de l'agent pathogène. Nul doute que cet insecte très vorace ingurgite plus de bactéries que l'altise qui ne grignote que le limbe des feuilles. On constate en effet que le doryphore, une fois contaminé au contact de plantes malades cause une forte infection alors que l'altise en est incapable. Les résultats démontrent que des doryphores désinfectés superficiellement, après leur passage sur des plantes malades, ont causé autant d'infection que ceux qui ne l'avaient pas été. Tout semble, en effet, indiquer qu'un grand nombre de bactéries, sinon la totalité, sont transportées par les organes internes de cet insecte. Il semble aussi que les insectes suceurs tels que les cicadelles, les aphrophores et les punaises ternes absorbent assez de bactéries pour causer une infection et même provoquer des symptômes de flétrissement.

Il est bon de noter que, pour chaque espèce d'insectes, l'inoculum provient d'un grand nombre d'individus et qu'il est impossible d'apprécier le nombre de bactéries transportées par chacun. Cependant, compte tenu d'un volume proportionnel d'inoculum, indépendamment du nombre d'insectes utilisés, le degré d'infection varie d'une espèce à une autre.

#### Méthode B

Cette méthode a pour but de déterminer si les insectes qui se nourrissent sur des plantes atteintes de flétrissure bactérienne peuvent, lors de leur séjour sur des plantes saines, y introduire l'agent de cette maladie.

D'abord des pucerons de l'espèce *Myzus persicae* (Sulz.), prélevés de plantes malades, furent déposés sur des feuilles détachées de plantes saines. Ces pucerons se sont nourris durant quatre à cinq jours sur des feuilles ainsi détachées, maintenues en état de turgescence par le trempage de leur pétiole dans l'eau. Comme il était difficile de déceler l'agent pathogène à l'aide de frottis ou de coupes microscopiques, ces feuilles furent lyophilisées et ensuite moulues très finement. Cette poudre, délayée dans quelques gouttes d'eau distillée, servit à inoculer des racines de plantes saines. De jeunes plants entiers, envahis par des pucerons contaminés, furent aussi lyophilisés un mois après le séjour de ces insectes et réduits en poudre. A titre de comparaison, des feuilles atteintes de la flétrissure furent traitées de la même façon afin d'apprécier l'efficacité de cette méthode.

Le pourcentage d'infection, obtenu de plantes inoculées avec la poudre de feuilles flétries, fut de 92 p. 100, et 25 p. 100 de ces plantes infectées ont

TABLEAU 2.—ÉTAT SANITAIRE DES PLANTES INOCULÉES AVEC POUDRE DE PLANTES LYOPHILISÉES AYANT HÉBERGÉ DURANT 4 JOURS DES INSECTES CONTAMINÉS

Espèce d'insecte	Plantes inoculées	Plantes flétries	Plantes infectées non flétries	Plantes saines
Doryphore	30	4	11	15
Altise	20	0	0	20
Cicadelle	24	0	2	22
Punaise terne	37	0	1	36
Puceron	39	0	2	37

flétri tandis que la poudre de feuilles et de tiges ayant hébergé les pucerons a donné respectivement 88 p. 100 et 66 p. 100 de plantes infectées dont aucune ne manifesta de symptômes.

Comme cette méthode de transmission semblait efficace, de nouveaux essais furent entrepris avec des pucerons et d'autres insectes du feuillage de la pomme de terre. En ce cas, des insectes tels que le doryphore, l'altise, la cicadelle, la punaise terne et le puceron, en contact avec des plantes flétries, furent déposés durant quatre jours dans des cages contenant des plantes saines. A l'approche de la maturité des plantes, des empreintes révélèrent la présence de bactéries à la base de la plupart de ces plantes, lesquelles furent ensuite lyophilisées et réduites en poudre. On a plus tard inoculé les racines de plantes saines avec cette poudre et diagnostiqué la maladie d'après les symptômes extérieurs au cours de la croissance et à l'examen microscopique des empreintes prélevées à la base des tiges. Les plantes témoins, n'ayant hébergé aucun insecte, étaient examinées de la même base.

### Résultats

Le tableau 2 permet de constater que la poudre des plantes qui ont hébergé l'altise, la cicadelle, la punaise terne et le puceron, ont causé une très légère infection tandis que celles qui ont hébergé le doryphore ont produit un plus fort degré d'infection. L'examen microscopique révéla la présence de *C. sepedonicum* dans la moitié des plantes inoculées, dont quatre montraient des symptômes de la flétrissure. Cette expérience confirme les résultats antérieurs, à savoir qu'au contact de tiges flétries, le doryphore peut non seulement transporter l'agent pathogène, mais qu'il peut aussi contaminer des tiges saines. Bien que la cicadelle et la punaise terne véhiculent un plus ou moins grand nombre de bactéries, elles ne paraissent pas les introduire facilement dans la plante. Comme les altises ne semblent pas être de bons agents de transmission de l'agent pathogène, il n'est guère surprenant qu'elles n'aient pas causé d'infection.

### Méthode C

Vu que les insectes du feuillage de la pomme de terre, notamment le doryphore, peuvent transmettre l'agent pathogène de la flétrissure, il convenait de déterminer si, une fois les tiges infectées, les bactéries pouvaient atteindre les tubercules au cours de la période de végétation en serre.

TABLEAU 3.—INFECTION DES PLANTES AYANT HÉBERGÉ DES INSECTES CONTAMINÉS SUR TRANCHES DE TUBERCULES MALADES

Espèce d'insecte	No. plantes soumises à l'infection	No. plantes ayant produit des tubercules infectés <sup>1</sup>
Doryphore	8	3
Altise	8	4
Cicadelle	6	3
Punaise terne	8	6
Aphrophore	8	5

<sup>1</sup>L'état sanitaire des plantes est déterminé par l'examen microscopique d'empreintes prises à la base des tiges issues de tubercules.

D'une part, on déposa, dans des cages où croissaient deux plantes saines, des insectes contaminés sur des tranches de tubercules malades. A ces plantes soumises à la contamination correspondaient des plantes témoins. Dans une autre expérience, on introduisit les mêmes espèces d'insectes dans des cages où croissaient une plante partiellement flétrie à côté de deux plantes saines. Dans chaque cas, le séjour des insectes dans les cages fut plus ou moins prolongé pour ne pas trop nuire à la croissance des plantes. Les tubercules issus de ces plantes furent entreposés et, après une période de repos de trois mois, de nouveau mis en terre afin de déceler des symptômes sur le feuillage ou la présence de bactéries à la base des tiges à l'aide de l'examen microscopique.

### Résultats

Les résultats inscrits au tableau 3 démontrent que le doryphore, l'altise, la cicadelle, la punaise terne et l'aphrophore, en présence d'une source de contamination, peuvent transporter l'agent pathogène et infecter des plantes saines. Le taux d'infection des plantes visitées par les insectes apparaît assez élevé si l'on considère que ces insectes, une fois contaminés sur des tranches de tubercules malades, n'avaient plus accès à cette source de contamination. Le degré d'infection obtenu à l'aide des altises est supérieur à celui qu'on obtint au cours des expériences antérieures. Cependant, il est bon de noter que cet insecte ne refuse pas de se nourrir au dépens du tubercule, tout comme les aphrophores et les punaises ternes qui sont des insectes polyphages, alors que le doryphore et la cicadelle ne se contaminent qu'occasionnellement sur des tranches de tubercules malades.

Les conditions de l'expérience en serre dont les résultats sont rapportés au tableau 4 s'apparentent davantage à celles que l'on retrouve dans les plantations de pommes de terre. En effet, la source d'infection demeure pour un temps à la disposition des insectes, une fois que ceux-ci sont introduits dans des cages contenant une plante flétrie à côté de deux plantes saines. Sous ces conditions, tous les insectes soumis à l'expérience ont réussi à transmettre l'organisme de la flétrissure, d'une plante malade à une plante saine. En effet, on constate que, parmi les plantes soumises à la contamination, un certain nombre ont produit des tubercules infectés. Parmi les plantes qui ont hébergé le doryphore, deux des six plantes infectées ont donné naissance à des tubercules qui, une fois mis en terre après une

TABLEAU 4.—INFECTION DES PLANTES CROISSANT DANS DES CAGES  
RENFERMANT DES INSECTES ET UNE PLANTE MALADE

Espèce d'insecte	No. plantes soumises à l'infection	No. plantes ayant produit des tubercules infectés <sup>1</sup>
Doryphore	10	6 <sup>2</sup>
Altise	8	1
Cicadelle	8	4
Punaise terne	8	8
Aphrophore	8	5

<sup>1</sup>L'état sanitaire des plantes est déterminé à l'aide de l'examen microscopique d'empreintes prises à la base des tiges issues des tubercules.

<sup>2</sup>De ces 6 plantes, deux ont donné naissance à des tubercules-filles qui, une fois mis en terre, ont produit des tiges flétries.

période de repos, ont produit des tiges qui manifestèrent des symptômes caractéristiques de la flétrissure. Dans les conditions de cette expérience, le doryphore seul réussit à transporter un nombre assez grand de bactéries pour infecter les tubercules au point d'obtenir des symptômes dans une génération subséquente. D'autre part, les cicadelles, les punaises ternes et les aphrophores, qui avaient été en contact avec un feuillage flétri, infectèrent les plantes saines alors que les altises se révélèrent moins aptes à la dissémination de cette maladie. Il est à signaler que les tubercules infectés, issus des plantes soumises à la contamination par les insectes autres que le doryphore, étaient exempts de symptômes externes de la flétrissure.

#### DISCUSSION

Les diverses méthodes mises à l'essai en serre indiquent que la plupart des insectes du feuillage de la pomme de terre ont pu transmettre l'agent pathogène de la flétrissure bactérienne, *C. sepedonicum*. En effet, l'infection a été obtenue par l'inoculation de plantes saines à l'aide d'une trituration d'insectes infectieux. Quelques-unes de ces plantes inoculées avec la trituration de doryphores, de cicadelles et d'aphrophores contaminés ont montré des symptômes de flétrissement. Cependant, ces résultats n'indiquent pas si les bactéries furent transportées sur les parties externes ou internes des insectes. Toutefois, l'on a observé que des plantes inoculées avec des doryphores infectieux, désinfectés avant d'être triturés, montraient autant de symptômes de flétrissement que les plantes inoculées avec des doryphores qui n'avaient pas été désinfectés. Il semble donc que le doryphore ait pu transmettre des bactéries à l'aide de ses organes internes. D'autre part, l'on ne sait pas si les bactéries se multiplient au sein de l'insecte puisqu'après un court séjour sur des plantes flétries, il est immédiatement trituré en une fine poudre servant à inoculer des plantes saines.

Dans des conditions identiques, l'infection a été obtenue par le libre mouvement des insectes d'une plante flétrie à une plante saine. En ce cas, les insectes avaient toujours accès à des plantes partiellement ou totalement flétries végétant sous cage, à proximité de plantes saines d'un stade de croissance variable. Si le doryphore infecta des plantes au point que les tubercules aient parfois donné naissance à des tiges atteintes de

flétrissement, il en fut autrement de la cicadelle, de la punaise terne et de l'aphrophore qui ont transmis la maladie au niveau du tubercule sans produire de tiges atteintes de flétrissement.

Si l'effet de masse constitue un facteur décisif dans la manifestation des symptômes, il semble bien que les bactéries transmises par des insectes autres que le doryphore d'une plante malade à une plante saine n'aient pas été assez nombreuses pour provoquer le flétrissement. Si toutefois l'on estime que l'inoculum transmis par la cicadelle, la punaise terne ou l'aphrophore fut assez abondant pour causer l'infection, il faudrait supposer que des conditions écologiques particulières n'étaient pas propices à l'expression des symptômes de la flétrissure.

L'unique but de ces expériences en serre était de vérifier si les insectes du feuillage de la pomme de terre pouvaient transmettre la flétrissure bactérienne. Les résultats obtenus nous incitent à poursuivre des travaux en plein champ afin de connaître si ces insectes sont vecteurs de la maladie sous des conditions naturelles.

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# MERCURY RESIDUES ON APPLE FRUIT AND FOLIAGE<sup>1</sup>

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## ABSTRACT

The disappearance of mercury from apple foliage and fruit sprayed with organo-mercury fungicides was investigated.

Most of the initial foliage deposit soon became water insoluble and spore germination tests on the surface of leaves demonstrated a rapid decline in fungitoxicity. The total amount of mercury on the foliage decreased rapidly in the first 2 weeks after spraying and more gradually in the succeeding 2 months.

The application of pre-cover sprays resulted in negligible fruit residues. The average harvest residue of fruit receiving an early cover spray was 0.05 p.p.m., of which the peel, pulp and seeds contained about 41, 57 and 2 per cent, respectively. There were no significant differences between residues in fruit sprayed on different dates.

## INTRODUCTION

Organo-mercury compounds have been used in Nova Scotia for several years, as eradicator or post-infection fungicides, to control apple scab caused by *Venturia inaequalis* (Cke.) Wint. Their use, however, has been confined to pre-cover sprays because of the zero tolerance for mercury in or on food products (1).

Ford and Burkholder (2) determined mercury residues on apple leaves and fruit at various intervals after applications of organo-mercury fungicides. They found residues of a few parts per billion on fruit at harvest and those on the foliage rarely exceeded 2 millimicrograms per leaf. Miller (5) studied the residues on apples in relation to the number of applications of organo-mercury fungicides. He found 6 to 8 applications resulted in residues of 0.04-0.07 p.p.m. in fruit harvested approximately 12 weeks after the last fungicide application. Stone *et al.* (9) reported measurable amounts of mercury in the skin and pulp of untreated apples and residues of approximately 0.05 p.p.m. where mercury fungicides were used in pre-cover sprays. Martin and Pickard (4) found some accumulation of mercury in the leaves but no build-up of surface deposits with 7 applications of a mercury fungicide. Fruit residues at harvest averaged about 0.05 p.p.m. of which approximately 18, 73 and 9 per cent were in the peel, pulp and core, respectively. Few studies have been reported on the relationship between time of application and mercury residues on fruit at harvest and apparently nothing has been reported on the rate of disappearance of organo-mercury fungicides from foliage.

Studies on the rate of disappearance of an organo-mercury fungicide from apple foliage and the magnitude of mercury residues on fruit at harvest were carried out during 1957 and 1958. The results obtained are given in this paper.

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## MATERIALS AND METHODS

On June 11, 1957, two 20-year-old Crimson Gravenstein trees were sprayed to "run-off" with Erad (phenyl mercury acetate) at a half-pint per 100 gallons. On the same date in 1958 four such trees were similarly sprayed. In both years leaf samples were taken from each tree at various intervals during the succeeding 3 months. The leaves were selected at random, but those on terminal growth were avoided. A disk, 2 cm. in diameter, was cut from each leaf and samples of 50 or 100 disks were used for analyses. Total and water soluble mercury residues were determined on the 1958 samples but only total mercury was determined in 1957.

In the determination of total mercury the leaf disks were digested with concentrated sulphuric acid and 30 per cent hydrogen peroxide, the procedure followed being essentially that of Polley and Miller (7). The mercury in the digest was then determined by the method of Rolfe *et al.* (8). Optical densities were measured at 490 m $\mu$  in a Beckman Model B spectrophotometer.

Soluble mercury was obtained by immersing the leaf disks in distilled water for 1 hour and providing occasional gentle agitation. The aqueous solution was filtered through glass wool and analysed by the procedure used for total mercury.

Spore germination tests on the surface of leaves from the Gravenstein trees sprayed with Erad were carried out in 1957 and 1958. At various intervals mature leaves were collected from the sprayed and unsprayed trees. Dust particles were removed from leaf surfaces with a blast of air, and disks 2 cm. in diameter were taken from the leaves and placed on microscope slides. The latter were then placed on glass racks in moist chambers. A drop of spore suspension of *Monilinia fructicola*, at 50,000 spores per ml. of distilled water containing 0.05 per cent orange juice as a stimulant, was placed at the centre of each disk. After 18 hours' incubation the covers were removed from the chambers and the spore suspension on the leaf disks was allowed to evaporate to dryness. Modifications of a technique developed by North (6) were used to remove the spores from the leaf disks. The latter were sprayed with a 2 per cent solution of cellulose acetate in acetone containing acid fuchsin to stain the spores. The film of cellulose acetate was allowed to dry and the disks were placed in a strong detergent which readily separated the film from the leaf disks. The films containing the spores were blotted on a paper towel and placed on microscope slides. A drop of acetone was added and a cover glass was immediately placed on the dissolved film. It was then possible to take germination counts on the spores. Counts were taken on 100 spores per disk. The spores from 6 to 8 disks were counted for each tree in the experiments.

In 1957, two Crimson Gravenstein trees of the type previously described were sprayed with Erad at a half-pint per 100 gallons on July 11; one of these trees received a second application on August 7. On these dates the apples were about 30 and 50 mm. in diameter, respectively. Fruit samples were taken at harvest and mercury residues were determined in the peel by using samples of 5 or 6 apples. Mercury residues were also



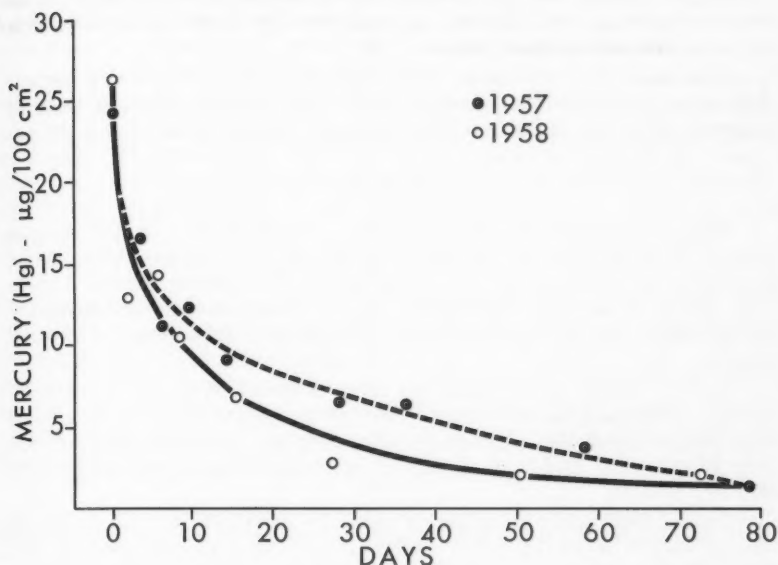


FIGURE 1. Decline of mercury residues on apple leaves.

determined in the peel of McIntosh apples from spray plots where the organo-mercury fungicides Phelam, Phix and Erad were applied as pre-cover sprays.

In 1958, the relationship between time of spray application and mercury residues at harvest was investigated. A randomized block design of three replicates of 3 Cortland trees per replicate was used. Erad at a half-pint per 100 gallons was applied to 1 tree in each replicate on June 17, 25 and July 3. The average diameter of the fruit at each spray date was 12, 17 and 23 mm., respectively, as determined from measurements made on random samples of 50 apples. Fruit samples were collected at harvest and mercury residues in the peel, pulp and seeds determined. Control samples of Cortland apples were not available in this block of orchard and were taken from another orchard.

The air-dried peel from 9 apples was placed in the digestion apparatus of Klein (3), 40 ml. of sulphuric acid added, and 25 ml. of nitric acid carefully introduced drop by drop. When vigorous oxidation ceased the mixture was heated and nitric acid gradually added. After heating for 3 to 4 hours and adding approximately 25 ml. of nitric acid a clear red solution was obtained. The digest was cooled, its acidity reduced to 1 N by the cautious addition of ammonium hydroxide, and 80 ml. of a 10 per cent hydroxylamine solution added. After the mixture had stood for at least 1 hour, mercury was determined by the method of Rolfe *et al.* (8).

Approximately 300 gm. of pulp was prepared by dicing one quarter from each of 9 apples. The pulp was air-dried for 1 week and analysed in the same manner as apple peel.

The seeds from 18 apples were air-dried and their mercury content determined by the method used for peel. The air-dried samples weighed approximately 4 gm. and reagent volumes were reduced accordingly.

### RESULTS

The mean values for total mercury residues found for leaf samples taken in 1957 and 1958 are presented in Figure 1. No measurable amounts of mercury were found on control samples. The results for the 2 years were almost identical. They show a rapid decline in residues during the first 10 days followed by a more gradual decline over a period of about 2 months.

Data on water-soluble mercury residues found on foliage in 1958 are given in Table 1. They show that a large proportion of the mercury applied soon became insoluble. Two hours after spraying the soluble fraction was only 17 per cent and after 8 days it was only 7 per cent of the total mercury present.

In the 1957 spore germination tests the inhibition on the surface of the leaves 2, 6 and 9 days after application of the fungicide averaged 100, 77 and 43 per cent, respectively. In 1958, at 0, 2, 5 and 8 days the inhibition was 100, 96, 42 and 20 per cent, respectively. There was considerable variation in the percentage inhibition found in any one germination test. This suggests that the fungicide available to the spores did not remain evenly distributed over the entire leaf.

The mercury residues in the peel of samples taken in 1957 are given in Table 2. Gravenstein apples, sprayed on July 11 or on July 11 and August 7, contained appreciable amounts of mercury at harvest, whereas fruit from McIntosh trees, which received only pre-cover sprays, contained mercury barely in excess of blank determinations.

Results of the experiment on mercury residues in relation to time of spraying are presented in Table 3. The initial amount of mercury on the

TABLE 1.—WATER SOLUBLE MERCURY ON APPLE LEAVES (Mean values for 4 trees)

Days between spraying and sampling	Soluble mercury (Hg) $\mu\text{g.}/100 \text{ cm.}^2$	Per cent of initial soluble mercury	Soluble as per cent of total mercury
0*	4.6	100	17
2	2.8	60	21
5	1.3	28	9
8	0.7	15	7

\*2 hours after spraying

surface of the apples was calculated by assuming that, on an area basis, initial residues on fruit and foliage were comparable. The calculations were based on residues on Gravenstein leaves.

Statistical analyses of peel, pulp, seed and total residues showed there were no significant differences between dates of spraying. The peel, which comprised about 13 per cent of the weight of the fruit, contained 41 per cent of the total residue. The pulp and seeds contained 57 and 2 per cent, respectively. On a fresh-fruit basis the average total residue of Cortland apples was 0.05 p.p.m.

### DISCUSSION

Following application of the organo-mercury fungicide Erad, foliage residues declined rapidly for approximately 10 days and gradually during the succeeding 2 months (Figure 1). The decline curves were almost identical in 2 consecutive years and were apparently little affected by rainfall. In 1958, 0.27 inches of rain fell 3 days after spraying and in 1957 no appreciable precipitation occurred for 7 days at which time there was 0.61 inches. Neither of these rains nor those occurring later during the

TABLE 2.—MERCURY RESIDUES IN APPLE PEEL (Mean values expressed on a fresh fruit basis)

Fungicide	Rate/100 gal.	Spray dates	Harvest date	Variety	No. of samples	Mercury(Hg) p.p.m.
Erad <sup>1</sup>	½ pt.	July 11	Sept. 18	Gravenstein	3	0.052
Erad	½ pt.	July 11, Aug. 7	Sept. 18	Gravenstein	3	0.125
Phelam <sup>2</sup>	1 lb.	May 14, 28, June 10	Sept. 24	McIntosh	4	0.004
Phix <sup>3</sup>	½ lb.	May 14, 28, June 10	Sept. 24	McIntosh	4	0.007
Erad	½ pt.	April 29, May 8, 16, 27, June 6	Sept. 27	McIntosh	4	0.003
Control			Sept. 27	McIntosh	2	0.003

<sup>1</sup> Erad, phenyl mercury acetate, 10% (Green Cross Insecticides, Montreal, Que.).

<sup>2</sup> Phelam, phenyl mercury dimethyl dithiocarbamate, 3% (F. W. Berk & Co., Ltd., London, England).

<sup>3</sup> Phix, phenyl mercury acetate, 22% (Chemley Products Co., Chicago, Ill.).

TABLE 3.—MERCURY CONTENT OF APPLES IN RELATION TO TIME OF SPRAYING (Mean values for 3 samples of 9 apples)

Spray date	Surface area cm. <sup>2</sup>	Calculated initial residue µg. Hg	Residue at harvest*			
			Peel	Pulp µg. Hg.	Seed	Total
June 17	37.3	9.7	28.5	40.5	1.3	70.3
June 25	85.5	22.5	26.4	40.4	1.4	68.2
July 3	152.1	40.0	26.9	33.9	1.4	62.2
Control			3.9	5.2	0.0	9.1

\* The differences between dates of spraying were not significant.

experimental work—5.5 inches in 1957 and 5.4 inches in 1958—caused any noticeable fluctuations in the rate of residue decline. In both years 50 per cent of the initial total mercury residue had disappeared before any appreciable precipitation occurred.

The soluble mercury fraction (Table 1) declined at an even greater rate than the total residue. This, and the results of the spore germination tests, indicated that, even without any weathering action by rain, the residue soon became non-fungitoxic. This suggests loss from foliage by volatilization or translocation.

Data for fruit residues (Table 2) show that pre-cover sprays resulted in only traces of mercury in the peel at harvest, whereas appreciable amounts occurred in fruit receiving cover sprays in July and August. These results are in agreement with those of Stone *et al.* (9).

The experiment on fruit residues in relation to date of spraying (Table 3) showed that Erad sprays applied on June 17, 25 or July 3 resulted in almost identical harvest residues even though the surface area of the apples increased four times between the first and last spray. It may be that, as the fruit and cuticle developed, less fungicide was absorbed, or, since the calculated initial surface residue was not large enough to account for the total harvest residue, mercury from other parts of the tree accumulated in the fruit. These results on fruit residues suggest that, with the present zero tolerance for mercury in or on food products (1), the use of organo-mercury sprays must remain confined to pre-cover applications.

The zero tolerance for mercury is perhaps unrealistic since in this and other work (4, 5, 9) mercury was found in apples which had not received mercury fungicides.

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# THE DRY MATTER DIGESTION IN VITRO OF FORAGE CROPS<sup>1</sup>

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## ABSTRACT

An artificial rumen technique was evaluated as a method of determining the dry matter digestion of forages. The digestibility estimates obtained in the spring were significantly correlated ( $r = 0.77^{**}$ ) with estimates obtained on these forages from a conventional digestion trial, whereas in the fall the coefficients were low and no longer significantly correlated ( $r = 0.49$ ) with the conventional trial data.

A medium maturing timothy polycross nursery was harvested at 10-day intervals throughout the first growth cycle. With the exception of the April 14th stage there were no significant differences in digestibility between the clones harvested in the early-leaf stage. Significant differences between selections were obtained in digestibility at later stages of maturity which may be a function of the leaf-stem ratio, the amount of leaf firing and thickness of the culms as well as changes in chemical composition. The per cent fructose content of this timothy herbage was significantly correlated ( $r = 0.78^{**}$ ) with the dry matter digestibility only at the first stage of cutting.

Changes in dry matter digestion using this artificial rumen technique were observed, with digestion estimates being higher during the spring and summer than during fall and winter.

The method of drying herbage for these *in vitro* digestion experiments was studied with the conclusion that there was a significant difference in favour of freeze-drying. The dry matter digestion coefficients of the freeze-dried herbage were comparable to those expected for herbage of such quality digested *in vivo*.

The use of an artificial rumen technique for estimating digestibility of clonal material shows promise for the plant breeder, providing certain precautions are taken. All lines to be screened should be included in a single trial to ensure maximum control of variables associated with technique. Samples should be uniformly processed, preferably freeze-dried, and digested with a single sample of rumen fluid.

## INTRODUCTION

A measurement of the digestibility of forages used as feed for ruminant livestock is usually obtained by feeding each forage to sheep or cattle in a conventional digestion trial. This method is expensive and the number of forages which can be tested is limited.

The "artificial rumen technique" has been presented by several workers as a method of obtaining a measure of the dry matter digestibility, cellulose digestion, or "quality" of forages. Kamstra (4) measured the rate of digestion *in vitro* of entire plants and of different cellulose fractions, of alfalfa, timothy, and orchard grass cut at three stages. He found that the rate of digestion of cellulose decreased by 50 per cent from the first

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to third cutting in orchard grass, and by 30 per cent in the case of the other two species; but the variation in the isolated cellulose fractions was small, thus showing the effect of lignin in reducing the rate of digestion of cellulose in whole samples. Pigden (6) obtained a correlation coefficient of "0.88" for digestibility of forages between the conventional digestion trial and an artificial rumen technique and proposed the latter as a method for evaluating forage quality.

To study the applicability of using an "artificial rumen technique" as a plant breeder's tool, it was proposed to obtain a measurement of the dry matter digestibility of different clones from selections within varieties of timothy, and to ascertain to what extent different stages of growth affected the digestibility.

### MATERIALS AND METHODS

In the standardization of the technique samples of forages of known digestibility were used. The timothy samples were obtained from a poly-cross nursery of 12 plant selections. This nursery, classified as "medium maturing", exhibited a good combination of desirable agronomic characters. Two replications of each clone were sampled from the nursery at weekly intervals starting April 14 and ending July 6 for analysis. The herbage was cut approximately 2 inches above ground level, and placed in plastic bags between pieces of dry ice for freezing.

Dry matter digestion was determined using the miniature artificial rumen technique of Huhtanen *et al.* (3). The rumen inoculum taken from a fistulated Holstein cow was prepared by a washed-cell method (2). Five hundred milligram samples of the test forages were weighed into previously dried and weighed semi-permeable visking sacs, then 10 millilitres of the prepared rumen inoculum were added, together with 25 millilitres of a simple phosphate buffer solution. This artificial rumen was then placed in a 4-ounce milk sample bottle which was filled with the buffer solution. The bottles, 60 per experiment, were placed in a constant temperature water bath for 24 hours at 39° C., the fermentation gases were periodically released, and the sac contents mixed. Following this digestion period the sacs were placed in a cold running water dialysis bath for 24 hours, then dried and weighed. The dry matter digestion was calculated, taking into account the weight of the inoculum added and using the following equation:

$$\% \text{ D.M. digested} = \frac{(\text{Original amount D.M. added}) - (\text{Ending weight of substrate})}{(\text{Original amount of substrate})} \times 100$$

Duplicate digestions of each test forage were made in all experiments.

### RESULTS AND DISCUSSION

The *in vitro* dry matter digestibility coefficients obtained from experiments conducted in June 1956 on forages supplied by Pigden were significantly correlated with the conventional trial dry matter digestion coefficients, the percentage digestible protein, and the percentage lignin



TABLE 1.—THE LIGNIN CONTENT, DRY MATTER, AND DIGESTIBLE PROTEIN COEFFICIENTS OF ELEVEN FORAGES COMPARED TO THEIR *in vitro* PER CENT DRY MATTER DIGESTIBILITY

Forage	% Dry Matter Digestion		% Digestible protein (6)	% Lignin content (6)
	Artificial rumen	Digestion trial (6)		
<i>Kochia scoparia</i>	70.5	68.9	17.7	7.7
Alfalfa	69.5	65.4	16.0	8.7
<i>Kochia scoparia</i>	60.8	55.8	7.8	10.5
<i>Kochia chidsii</i>	49.2	55.4	6.9	11.8
Bromegrass	44.5	63.6	9.2	9.5
Intermediate wheat	43.4	61.3	6.3	10.4
Russian wild ryegrass	42.7	60.4	9.9	11.4
Oat hay	43.2	59.4	7.4	9.4
Canadian wild ryegrass	41.4	58.4	8.0	10.9
Brome straw	46.0	57.2	5.4	10.7
Marsh hay	39.1	53.9	2.5	13.7
S.E.M.	1.5			
L.S.D. (P = .05)		3.5		
Coefficients of correlation between art. rumen dig. and:		0.77**	0.87**	-0.68*
*Significant at (P = 0.05)				
**Significant at (P = 0.01)				

(6) Pigden, W. J. 1954.

TABLE 2.—PERCENTAGE DRY MATTER DIGESTIBILITY BY STAGES OF FREEZE-DRIED TIMOTHY HERBAGE, ARTIFICIAL RUMEN

Selection	Clone No.	April 14	April 24	May 3	May 12	May 21	May 31	June 9	June 18	June 27	July 6
Growth stage*		L	L	L	L	L	L	EB	FB	F	S
Drummond	10	63.7	71.3	68.8	74.2	60.0	63.6	56.2	50.5	50.5	41.0
Tuarmo	11	68.6	72.3	71.0	71.9	62.7	62.1	57.9	49.3	52.8	46.2
Climax	12	63.4	75.5	72.2	70.6	54.7	54.2	50.1	44.7	47.9	36.6
Milton	13	66.9	73.9	66.7	74.0	58.4	56.7	56.9	44.1	48.9	40.2
Lorain	14	64.5	74.7	70.5	73.1	61.4	55.9	57.4	49.3	48.7	42.3
Acc. 1934	15	76.1	71.9	71.1	73.6	64.5	53.7	53.0	49.2	51.7	39.1
Hopkins	16	65.6	73.5	70.5	74.0	61.4	63.8	58.4	48.6	46.5	42.7
Bottnia	17	65.8	72.6	73.9	70.7	59.2	59.8	59.2	52.0	55.7	44.0
Acc. 1930	18	66.8	72.1	68.0	71.2	59.8	55.5	57.9	50.8	51.4	42.9
Medon	19	71.5	74.0	70.5	68.5	57.6	57.6	56.5	48.9	51.9	40.1
S-48	20	62.0	72.7	67.2	70.2	61.4	57.5	51.6	52.3	51.5	41.7
Omnia	21	67.7	74.5	70.4	72.2	57.6	62.9	56.8	45.7	54.9	39.0
S.E. in %		3.24	—	—	—	—	3.6	3.05	4.14	3.18	5.27
S.E.M. =		2.16	NSD	NSD	NSD	NSD	2.11	1.71	2.01	1.61	2.18

\*L = Leaf EB = Early Boot FB = Full Boot F = Flower S = Seed Stage

content. These supplemental data are presented in Table 1 in support of the premise that any *in vitro* digestion technique must show a close relationship with the conventional digestion trial, and if the measured variable will change with the stage of growth of the plant, then it must also be correlated with the digestible protein and lignin contents.

The dry matter digestion estimates by stages of growth of the freeze-dried timothy herbage are presented in Table 2. Some of the differences at the April 14th stage were probably due to the presence of old growth



TABLE 3.—DRY MATTER DIGESTION AND FRUCTOSE CONTENT OF CLONE 19 WHEN DETERMINED ON OCTOBER 16, 1956

Date of harvest		Dry matter digestion %	Fructose content %	Stage of growth
April	14	68.4	13.4	Vegetative
	24	67.8	16.6	Vegetative
May	3	69.7	10.8	Vegetative
	12	60.9	3.8	Vegetative
	21	60.3	7.4	Vegetative
	31	55.3	3.2	Vegetative
June	9	52.9	5.3	Early boot
	18	51.8	4.2	Full boot
	27	44.9	6.0	Flower
July	6	48.1	11.0	Tips burned seed stage

D.M. Digestion S.E.M. = 0.81

which was difficult to separate out of the short, new season's growth. However, clones 15 and 19 at this date had a very high fructose content (22 per cent) which undoubtedly contributed to the high digestibility estimate since the percentage fructose content was related to the dry matter digestion estimate ( $r = 0.78^{xx}$ ) at this early stage.

Beginning with late flower through the seed stage, there were differences in the dry matter digestion which could have been a function of the leaf-stem ratio, the amount of leaf firing, and the thickness of the culms as well as changes in chemical composition. There appears to be a decided drop in the dry matter digestibility estimates between the May 12th and the May 21st cuttings. This drop was coincident with early culm formation. When all stages of any one selection were digested in the same experiment in order to exclude differences due to inoculum used, this apparent reduction in digestion was less evident.

TABLE 4.—ESTIMATES OF THE PER CENT DRY MATTER DIGESTIBILITY ON REPLICATE 48 AS DIGESTED ON JULY 19 AND DECEMBER 1, 1956

Clone No.	Dry matter digestibility estimates	
	July 19	December 1
10	75.7	64.5
11	72.1	63.1
12	66.6	63.8
13	70.7	63.2
14	74.5	68.7
15	75.7	65.2
16	75.5	67.1
17	71.5	68.8
18	74.0	63.0
19	65.7	61.5
20	72.0	65.7
21	70.2	68.8

S.E.M. Clones = 1.7

S.E.M. Dates = 1.35

TABLE 5.—A COMPARISON OF THE DRY MATTER DIGESTION COEFFICIENTS OF ELEVEN FORAGES AS DETERMINED DURING THE MONTH OF OCTOBER AND THE MONTH OF JUNE

Forage	% Dry matter digestion artificial rumen		Conventional trial (6)
	Spring (June)	Fall (October)	
<i>Kochia scoparia</i>	70.5	67.0	68.9
Alfalfa	69.5	72.4	65.4
<i>Kochia scoparia</i>	60.8	59.5	55.8
<i>Kochia childsii</i>	49.2	50.5	55.4
Bromegrass	44.5	37.3	63.6
Intermediate wheat	43.4	35.5	61.3
Russian wild ryegrass	42.7	34.3	60.4
Oat hay	43.2	36.2	59.4
Canada wild ryegrass	41.4	32.3	58.4
Brome straw	46.0	34.8	57.2
Marsh hay	39.1	33.5	53.9
S.E.M.	1.5	1.1	

Dry matter digestion and percentage fructose in clone 19 are presented in Table 3. Inconsistencies were evident between the results obtained on October 16 when compared to the estimates obtained when this material was cut and dried (Table 2). In general, there was a slightly lower estimated digestibility on all samples digested throughout the late fall and winter. No relationship was found between the percentage fructose content and the *in vitro* dry matter digestion.

To test whether there was a significant reduction in the dry matter digestibility from the summer to late fall, an experiment was conducted using herbage cut May 12 and digested July 19 compared with the same herbage digested December 1. In Table 4 the results indicate that there was a difference ( $P > .01$ ) between dates at which the digestion experiment was conducted. The interaction of selection  $\times$  date was not significant as was the within duplicate samples variance. Similarly, when the forages of known digestibility were used as the substrate in late October, it was found that the grass sample digestion estimates had been reduced from the spring values. This comparison is presented in Table 5. The reduction was of sufficient magnitude to result in the simple correlation coefficient between the artificial rumen and conventional trial coefficients falling from 0.77 to 0.49. However, a highly significant  $r$  value of 0.98 was still obtained between the spring and fall digestion estimates. Changes in diurnal, daily, and seasonal fluctuations in the concentration of "free" rumen bacteria and in rumen pH have been reported by Nottle (5), which may be sufficient to account for the lowered digestion coefficients obtained in the fall. Whether this reduction in dry matter digestibility was due to decreased activity of the rumen microflora or changes in the stored herbage was not investigated. Such discrepancies as these are not uncommon when using *in vitro* digestion techniques. Baumgardt (1) found that any change in the diet of the animal used as a source of inoculum, the time of collection of the rumen fluid, the method of preparation, or the length of the digestion period would invalidate sequential experiments.

TABLE 6.—DRY MATTER DIGESTION ESTIMATES, IN PER CENT, ON FREEZE-DRIED AND OVEN-DRIED TIMOTHY

Clone No.	Freeze-dried	Oven-dried 170° F.
10	64.5	51.4
11	63.2	51.2
12	63.8	49.7
13	63.2	48.9
14	68.7	53.6
15	65.2	48.1
16	67.1	53.3
17	68.8	47.1
18	63.0	45.7
19	61.5	49.8
20	65.7	52.2
21	68.8	52.5

S.E.M. of experiment = 1.23

TABLE 7.—DRY MATTER DIGESTION ESTIMATES, IN PER CENT, ON TUARMO THROUGHOUT THE 1956 SEASON

Date of cutting	Freeze-dried	Oven-dried 170°F.
April 14	66.9	55.0
24	66.3	55.2
May 3	69.6	60.4
12	62.1	46.3
21	63.4	44.7
31	58.9	38.3
June 9	55.3	39.4
18	54.2	39.7
27	47.3	39.6
July 6	48.8	42.3

S.E.M. of experiment = 1.65

Method of drying the sampled herbage was also a source of variation. A comparison was made between freeze-dried herbage and that dried at 170° F. Table 6 presents means of duplicate samples of the May 12th stage of cutting. Considering selections and treatments as fixed, the analysis of variance shows differences between treatments ( $P > .01$ ) and selections ( $P > .05$ ). The interaction variance of selection x treatments was not significant, indicating that for comparative purposes the estimates on oven-dried herbage might be acceptable. However, such a reduction in digestibility could nullify the seasonal variations and decline as exemplified in a test comparing seasonal cuts out of the selection from the timothy variety Tuarmo. Table 7 presents these data and shows that differences ( $P > .01$ ) between treatments and dates of harvest occurred. Although the interaction of treatments x dates of cutting was not significant, oven drying tended to decrease differences between stages of growth. This selection out of Tuarmo was the most desirable as to leafiness, erectness, and retention of leaf.

Even at the July 6th cutting there was only slight leaf firing and as the ratio of leaf to stem was favourable, its high dry matter digestibility as measured by the artificial rumen technique was retained.

The use of the miniature artificial rumen for estimating digestibility of clonal material by the plant breeder shows promise. Present evidence indicates that the investigator should include all of the lines to be screened for digestibility into a single trial so as to ensure maximum control of the variables associated with the technique. It is imperative that samples for digestion studies be processed uniformly—preferably freeze-dried—and that a single sample of rumen fluid be used in any one trial. The reproducibility of the results is satisfactory and correlations with the conventional digestion trial are high.

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# VARIETY, FERTILIZER, MANAGEMENT INTERACTIONS IN ALFALFA

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## ABSTRACT

An experiment was conducted for the purpose of studying over a 4-year period the interactions to be found among four alfalfa varieties differing in winter hardiness and wilt reaction, six fertilizer treatments differing quantitatively and qualitatively, and three management systems differing in the frequency and timeliness of cutting.

In the second crop year hardiness was the main factor in determining both yields and stands; bacterial wilt was of somewhat less importance. In the third crop year wilt became the main factor. Varieties differed significantly in their reaction to potash, to phosphorus and to the severity of cutting treatments. Phosphorus helped the winter-hardy varieties more than the non-hardy, whereas potash was of greater benefit to the winter-susceptible varieties.

The management system which included a September clipping was detrimental to all varieties and particularly to the less hardy ones, DuPuits and Ranger. Only with the hardy varieties, Vernal and Grimm, was it feasible to attempt to compensate by the addition of fertilizers for the mismanagement involved in untimely cutting.

## INTRODUCTION

During the last 5 years at least two new factors in alfalfa production have prompted a very considerable change in the philosophy of management of this crop in Ontario. The first was the realization that bacterial wilt is of frequent occurrence in many sections of the province. The second was the introduction into Ontario of new alfalfa varieties possessing wide differences in such agronomic characteristics as growth potential, date of maturity, winter hardiness and bacterial wilt resistance.

It seemed probable that these new varieties might differ from the old in their reaction to Ontario farm practices and an experiment was designed to study some first- and second-order interactions between varieties, fertilizers and cutting managements. In particular it was thought desirable to determine whether fertilizer applications could overcome the detrimental effects that were expected to ensue from too frequent or untimely cutting treatments and whether this remedial action might be applicable to all varieties.

## LITERATURE REVIEW

An understanding of the perennial nature of the alfalfa plant and of its growth habits and in particular of the way it prepares for winter is basic to an intelligent management practice. Chiefly instrumental in acquiring and disseminating such knowledge in the third and fourth decade of this century were such research workers as: Graber (3), Grandfield (4), Janssen (7), Jones (8), Rather (10), Silkett (11), and Williard (14). Their studies have been supplemented by those of later workers: Blaser (2), Graumann (5), Gross (6), Nielsen (9), and others. Still others have concentrated on

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the role and effect of fertilizer supplements. Bear (1), Stivers (12), and Wang (13), to mention only a few, are included in this group. Excellent reviews of all phases of these studies have been provided by a long list of workers to which Gross is the latest addition. The detailed findings of the individual workers will not be listed here but instead a summary will be made of those main principles of alfalfa management which have been established by these many workers and which are pertinent to the present study.

In the late summer and early fall an alfalfa plant in the natural state produces a supply of crown buds and also stores in its crown and roots a quantity of reserve food. The number of crown buds is adequate to provide shoots not only for the hay crop of the ensuing year but for several aftermath cuts as well. The reserve food enables the plant to better withstand the winter temperatures and in addition it furnishes material for the growth of new shoots the following year. Interference with this natural process, an example of which is September defoliation, results in increased winter killing and decreased yields the following season.

In the spring only a portion of the crown buds develop immediately into aerial shoots. The rest remain dormant until the first growth has either ripened or has been removed, following which a portion of the remainder develop. This process of defoliation followed by regrowth may be repeated several times during the summer. Each cycle reduces both the number of crown buds and the quantity of reserve food. Too frequent cutting may exhaust the plant's resources to such an extent that it may die either from lack of crown buds with which to produce new shoots or from lack of reserve food with which to feed the new shoots.

To reduce the risk to the alfalfa plant attendant on low fertility levels phosphorus and potash are frequently added. These two fertilizers play distinctly different roles in plant nutrition. Phosphorus, being an essential constituent of nucleoproteins, is needed for the construction of new cells. A deficiency of it results in poorer growth. Potash plays an important role as a catalyst in carbohydrate metabolism. When it is in short supply, as frequently happens in lighter textured soils, the alfalfa plant tends to become spindly and succulent and to have reduced resistance to diseases. Stands thin out rapidly.

#### MATERIALS AND METHODS

As shown below, the varieties used each represented a different combination of the two factors, winter hardiness and bacterial wilt resistance.

	Hardy	Less hardy (non-hardy)
Wilt-resistant.....	Vernal	Ranger
Wilt-susceptible.....	Grimm	DuPuits

The site chosen was a sandy loam, slightly alkaline soil, medium low in both phosphorus and potassium. Bacterial wilt was known to be present. The fertilizer treatments, listed below, were included only for the purpose of providing information on interactions between fertilizers, varieties and cutting treatments. The quantities shown were applied twice; once in the spring of the first crop year, and again in the fall of the same year.



Treatment Designation	Treatment
O	Check, i.e. no fertilizer
P	35 lb. $P_2O_5$
K	72 lb. $K_2O$
PK	35 lb. $P_2O_5$ + 72 lb. $K_2O$
2PK	70 lb. $P_2O_5$ + 144 lb. $K_2O$
M	10 tons farmyard manure

Three different cutting treatments, sometimes referred to as *mild*, *average* and *harsh* respectively, were used. These were designated as:

$C_2$  — cut in June, August, 1955-57

$C_3$  — cut in June, August, mid-late October, 1955-57

$C_4$  — cut in June, August, mid-late September, mid-late October, 1955-57.

The experiment was set up as a split-plot with four replicates. The main, sub-plots and sub-sub-plots were varieties, fertilizers and cutting treatments respectively, the size of the smallest plots being 25 feet x 5 feet. The seed was broadcast-sown in August, 1954, with a light starter application of fertilizer. Establishment was good. Plant counts were made in the O, P, K, PK plots managed under the  $C_2$  and the  $C_4$  cutting systems. These were made each year in the late summer or early fall. Two samples, each sized 2.8 square feet, were taken from each plot and permanent quadrats were used for this purpose. Weeds were removed by hoeing.

## RESULTS AND DISCUSSION

Forage yield and population density in an established stand of alfalfa are influenced by many factors among which are growth potential, winter hardiness, bacterial wilt reaction, soil fertility and management practices. All these factors, constituting sources of variation in the experiment, will be discussed in the following sections.

### *Varieties and Cutting Management*

Since it was not until the end of 1955 that the preparatory stages of the experiment, i.e., all treatments applied at least once, were completed, the 1955 data will be mentioned only briefly.

In 1955 plot stands were both uniform and good, averaging over 14 plants per square foot. In yields, however, there was variation, the ranking of the varieties being DuPuits, Vernal, Ranger and Grimm. The differences in yield were sufficiently great to indicate that the growth potential of DuPuits is high, that of Vernal and Ranger is good, and that of Grimm somewhat lower.

In 1956 the effect of the degree of winter hardiness and to a lesser extent of wilt reaction of the four varieties was showing up. This is illustrated in Table 1 where plant populations are shown for all varieties under four different fertilizer and two different cutting treatments. DuPuits and Ranger had poorer stands than Vernal and Grimm and this was particularly noticeable under harsh cutting management  $C_4$ . From this association of varieties it may be concluded that lack of winter hardiness was the main contributing factor. Analysis of variance for yield (Table 2, (item A)) lends support to this thesis. Although for all varieties the yield gradings were  $C_3 > C_2 > C_4$ , the differences between treatments varied greatly



TABLE 1.—NUMBER OF PLANTS PER SQUARE FOOT FOR VARIETY, YEAR, FERTILIZER AND CUTTING TREATMENTS

Variety	Year	O Check		K K <sub>2</sub> O		P P <sub>2</sub> O <sub>5</sub>		PK P <sub>2</sub> O <sub>5</sub> +K <sub>2</sub> O		Mean		Variety mean
		C <sub>2</sub>	C <sub>4</sub>	C <sub>2</sub>	C <sub>4</sub>	C <sub>2</sub>	C <sub>4</sub>	C <sub>2</sub>	C <sub>4</sub>	C <sub>2</sub>	C <sub>4</sub>	
DuPuits	1954	22.0	25.0	25.0	25.0	27.0	23.0	20.0	24.0	23.5	24.3	23.9
	1955	13.0	16.0	16.0	16.0	15.0	14.0	12.0	13.0	14.0	14.8	14.4
	1956	6.4	2.4	7.9	5.9	5.9	3.1	6.9	3.8	6.8	3.8	5.3
	1957	1.7	0.1	4.2	0.7	2.0	0.7	2.5	1.5	2.6	0.8	1.7
Ranger	1954	22.0	24.0	24.0	28.0	22.0	31.0	25.0	23.0	23.3	26.5	24.9
	1955	13.0	14.0	14.0	14.0	12.0	14.0	12.0	12.0	12.8	13.5	13.2
	1956	7.2	4.5	7.2	6.1	6.1	6.4	5.9	4.1	6.6	5.3	6.0
	1957	5.5	3.2	5.0	5.0	4.7	3.7	4.5	3.0	4.9	3.7	4.3
Vernal	1954	24.0	25.0	22.0	21.0	17.0	22.0	24.0	22.0	21.8	22.5	22.2
	1955	15.0	16.0	14.0	15.0	12.0	17.0	14.0	13.0	13.8	15.3	14.6
	1956	8.7	7.7	8.3	9.7	6.8	6.6	9.9	7.8	8.4	8.0	8.2
	1957	6.7	6.1	6.5	6.5	4.2	4.0	7.7	7.0	6.3	5.9	6.1
Grimm	1954	22.0	24.0	16.0	18.0	19.0	25.0	20.0	21.0	19.3	22.0	20.7
	1955	15.0	13.0	13.0	13.0	14.0	15.0	14.0	15.0	14.0	14.0	14.0
	1956	7.0	3.6	7.2	8.2	7.6	6.8	9.2	4.9	7.8	5.9	6.9
	1957	1.2	1.5	3.1	2.2	3.0	2.7	2.0	2.0	2.5	2.1	2.3
Mean	1954	22.5	24.5	21.8	23.0	21.3	25.3	22.3	22.5	22.0	23.8	22.9
	1955	14.0	14.8	14.3	14.5	13.3	15.0	13.0	13.3	13.7	14.4	14.1
	1956	7.3	4.6	7.7	7.5	6.6	5.7	8.0	5.2	7.4	5.8	6.6
	1957	3.8	2.7	4.7	3.6	3.5	2.8	4.4	3.4	4.1	3.1	3.6

TABLE 2.—TABLE OF VARIANCES FOR 1956, 1957 YIELDS

Item	Source of variance	Degrees of freedom	Mean square	
			1956	1957
A B AB	Variety	3	2.61**	49.84**
	Hardiness (hardy vs. non-hardy)	1	2.11**	17.99**
	Wilt resistance (resistance vs. susceptibility)	1	1.05	131.17**
	Hardiness × wilt	1	4.67**	.36
C D E	Fertilizer	5	4.25**	4.78**
	Fertilizers vs. check (P,K,PK,2PK,M vs. O)	1	8.22**	11.55**
	Potash (K+PK) vs. (O+P)	1	5.72**	7.12**
	Phosphorus (P+PK) vs. (O+K)	1	0.32	0.03
G H	Hardiness × phosphorus	1	1.12*	2.38*
	Cutting management	2	11.66**	19.55**
	Harsh vs. medium (C <sub>4</sub> vs. C <sub>2+3</sub> )	1	14.64**	10.04**
	Mild vs. average (C <sub>2</sub> vs. C <sub>3</sub> )	1	8.69**	29.07**
I J K L M	Cuts × fertilizer	10	0.24	0.31**
	Harsh × fertilizer (see G and C)	1	1.50**	0.09
	Harsh × phosphorus	1	0.17*	0.14
	Harsh × potash	1	0.84**	0.23
	Medium × fertilizer (see H and C)	1	0.39**	0.27
	Medium × potash	1	0.38**	0.23
N O P Q R	Cuts × varieties	6	0.76**	1.18**
	Harsh × hardiness (see G and A)	1	3.28**	0.99**
	Harsh × wilt (see G and B)	1	0.31**	1.17**
	Medium × hardiness (see H and A)	1	0.66**	2.08**
	Medium × wilt (see H and B)	1	0.06	1.99**
	Medium × hardiness × wilt	1	0.03	0.84**
S T	Cuts × fertilizer × variety	30	0.11**	0.16
	Harsh × phosphorus × hardiness	1	0.31**	0.00
	Harsh × potash × hardiness	1	0.34**	0.07

with the variety. Vernal's yield (see Table 3) under the C<sub>4</sub> treatment was only 0.2 tons less than the average obtained under the C<sub>2</sub> and C<sub>3</sub> systems and Grimm's was 0.3 less, whereas Ranger's was 0.6 and DuPuits' 0.7 less. It is seen that the less hardy varieties suffer a larger decrease attributable to untimely management than the hardy varieties but that within

each of the hardy and non-hardy groups the wilt-susceptible types provide a bigger drop in yield than the resistant types do. These differences, which are highly significant (items N,O), indicate that in 1956 both hardiness and wilt reaction are factors in yield reduction. The same trend is seen when the two mild treatments, C<sub>2</sub> and C<sub>3</sub>, are compared, although in this comparison the differences are smaller and only hardiness is interacting significantly with cutting management (item P).

By 1957 the situation had become reversed so that wilt had now become a factor of greater importance than hardiness. This is shown by the greatly reduced stands of the wilt susceptible varieties, DuPuits and Grimm (see Table 1). Ranger, the non-hardy fraction of whose population had largely been killed previously, suffered a much smaller decline and Vernal even less. Yield trends followed the same pattern as plant stands and differences due to hardiness and to wilt were both highly significant (items A,B). The differences between medium and harsh cutting treatment yields for

TABLE 3.—1956-57 YIELDS IN TONS OF DRY MATTER PER ACRE

	O Check			K K <sub>2</sub> O			P P <sub>2</sub> O <sub>5</sub>			PK K <sub>2</sub> O + P <sub>2</sub> O <sub>5</sub>		
	C <sub>2</sub>	C <sub>3</sub>	C <sub>4</sub>	C <sub>2</sub>	C <sub>3</sub>	C <sub>4</sub>	C <sub>2</sub>	C <sub>3</sub>	C <sub>4</sub>	C <sub>2</sub>	C <sub>3</sub>	C <sub>4</sub>
1956												
DuPuits.....	2.6	3.0	1.7	2.5	3.3	2.8	2.6	2.6	1.9	2.8	3.3	2.4
Ranger.....	2.6	2.8	1.7	2.4	3.2	2.3	2.4	2.8	1.9	2.4	3.0	2.0
Vernal.....	2.7	2.7	2.3	2.7	3.2	2.7	2.6	2.7	2.6	2.9	3.5	3.2
Grimm.....	2.3	2.4	1.7	2.7	2.6	2.3	2.3	2.8	2.4	2.7	2.9	2.5
Mean 1956....	2.5	2.8	1.8	2.6	3.1	2.5	2.5	2.7	2.2	2.7	3.2	2.5
Mean 1957....	1.9	1.6	1.0	2.4	2.1	1.6	1.9	1.7	1.5	2.5	1.9	1.4
1957												
DuPuits.....	1.2	.8	.1	2.1	.9	.8	1.2	.4	.5	2.1	.8	.3
Ranger.....	2.5	2.3	1.4	2.6	2.7	2.2	2.0	1.8	1.8	2.9	2.1	1.5
Vernal.....	2.9	2.5	2.0	3.1	3.2	2.6	2.7	2.6	2.3	3.1	3.1	2.8
Grimm.....	1.0	.8	.4	1.8	1.5	.9	1.9	1.9	1.2	1.8	1.5	.9

	Fertilizer means						Cutting means				Variety mean
	O	K	P	KP	2KP	M	C <sub>2</sub>	C <sub>3</sub>	C <sub>2</sub> +3		C <sub>4</sub>
									2		
1956											
DuPuits.....	2.4	2.8	2.3	2.8	3.0	2.9	2.7	3.2	2.9	2.2	2.7
Ranger.....	2.4	2.6	2.4	2.5	2.7	3.0	2.5	3.1	2.8	2.2	2.6
Vernal.....	2.6	2.9	2.6	3.2	3.4	3.4	2.9	3.3	3.1	2.9	3.0
Grimm.....	2.1	2.5	2.5	2.7	3.0	3.1	2.6	2.9	2.7	2.4	2.6
Mean 1956....	2.4	2.7	2.5	2.8	3.0	3.1	2.7	3.1	2.9	2.4	2.7
Mean 1957....	1.5	2.0	1.7	1.9	2.2	2.3	2.4	2.0	2.2	1.5	2.0
1957											
DuPuits.....	.7	1.3	.7	1.1	1.3	1.3	1.9	.9	1.4	.4	1.1
Ranger.....	2.1	2.5	1.9	2.2	2.4	2.9	2.7	2.4	2.5	1.9	2.3
Vernal.....	2.5	3.0	2.5	3.0	3.4	3.0	3.1	3.0	3.0	2.6	2.9
Grimm.....	.7	1.4	1.7	1.4	1.7	2.0	1.9	1.5	1.7	1.0	1.5

TABLE 4.—HARDINESS  $\times$  CUTTING TREATMENT  $\times$  POTASH INTERACTION, 1956  
Yield in Tons of Dry Matter per Acre

	Hardy		Non-Hardy		Mean
	$C_{2+3}$	$C_4$	$C_{2+3}$	$C_4$	
	2		2		
Potash (K, PK)	2.9	2.7	2.9	2.4	2.7
No potash (O,P)	2.6	2.2	2.7	1.7	2.3

DuPuits, Grimm, Ranger and Vernal were 1.0, 0.7, 0.6, 0.4 tons per acre respectively. Smaller differences are noted when the  $C_2$  and  $C_3$  treatments are compared. In 1957 the  $C_2$  treatment for the first time outyielded the  $C_3$ . All interactions between cutting treatments, hardiness and wilt reaction (items N,O,P,Q,R) were highly significant.

Over the 3-year period, 1955-57, the  $C_2$  and  $C_3$  treatments provided about the same total yield and averaged 30 per cent more than the 4-cut treatment. Clearly, under Ontario conditions, cutting of alfalfa in September is extremely detrimental to future yields. If, however, September grazing or cutting cannot be avoided, the use of a variety of Vernal's type minimizes the damage.

#### Fertilizer Interactions

Table 4 shows that potash was helpful in all circumstances in increasing yields, being responsible on the average for an extra 0.4 tons per acre. Under the stress of untimely cutting management it increased yields by an average of 0.6 tons compared with 0.2 tons under mild cutting treatments and in the  $C_4$  treatment potash helped the non-hardy more than the hardy types. These interactions were all significant (items D,K,M,T). Reference to Table 1 provides the explanation. It shows that potash had an important salutary effect in maintaining stands and that it was most effective where the need was greatest, i.e., under severe management and for less hardy varieties. The beneficial effect of this fertilizer was still evident in 1957 (item D) but significant interactions had largely disappeared.

Phosphorus gave much more variable results than potash did and was much less effective than potash in providing help where the need was greatest. The over-all increase in yield (see Table 5) attributable to phosphorus was small and not significant. It increased the yield of the hardy varieties by an average of 0.3 tons and decreased that of the non-hardy types by 0.1 tons, a significant interaction (item E). Most of the extra yield for the hardy types resulted from the 0.5-ton increase shown under the  $C_4$  treatment, hence the highly significant phosphorus  $\times$  hardiness  $\times$  cutting management interaction, (item S).

Reference to Table 1 shows that this fertilizer was much less effective in maintaining stands than was potash and that it was, in fact, frequently responsible for reducing stands below the level found in the check. More-

TABLE 5.—HARDINESS  $\times$  CUTTING MANAGEMENT  $\times$  PHOSPHORUS INTERACTION, 1956

Yield in Tons Dry Matter per Acre

	Hardy			Non-hardy			Mean
	C <sub>2+3</sub>	C <sub>4</sub>	Mean	C <sub>2+3</sub>	C <sub>4</sub>	Mean	
	2			2			
Phosphorus (P,PK)	2.8	2.7	2.7	2.7	2.0	2.3	2.5
No phosphorus (O,K)	2.7	2.2	2.4	2.8	2.1	2.4	2.4

TABLE 6.—A COMPARISON OF VARIETY YIELDS UNDER THREE MANAGEMENT-FERTILIZATION SYSTEMS

Yield in Tons of Dry Matter per Acre

Variety	Well managed, not fertilized	Badly managed, not fertilized	Badly managed, well fertilized
DuPuits 1956	2.8	1.7	2.4
1957	1.0	0.1	0.4
Ranger 1956	2.7	1.7	2.5
1957	2.4	1.4	2.2
Vernal 1956	2.7	2.3	3.3
1957	2.7	2.0	3.0
Grimm 1956	2.3	1.7	2.9
1957	0.9	0.4	1.4

over, yields were frequently unrelated to population density. Vernal's yields under phosphate treatment throughout the experiment were substantially equal to those for the check whereas the populations were considerably lower. Also, Ranger's yields in 1955 were lower for phosphate than for check but the populations were similar and sufficiently high to provide perfect ground cover. These data suggest that, in contrast to potash, the contribution of phosphorus to yield—and, as may be seen from Table 3, this may be negative—is direct and not expressed through plant stands.

It had been pointed out in the section under "Varieties and Cutting Management" that untimely cutting had had a markedly harmful effect on both plant stands and plot yields. Likewise, it had been demonstrated that potash almost invariably, and phosphorus frequently, had beneficial effects on plant stands and plot yields. One of the objects of this experiment was to study the interplay of these two opposing forces and, in fact, to determine whether it was possible by the use of fertilizers to overcome the detrimental effects of mismanagement. The two fertilizers which lent themselves most readily to this problem were the double dose of phosphorus and

potash, i.e. 2PK, and the farmyard manure, M. The increases in yield provided by these two were of the order of 0.6-0.8 tons per year. These increases were highly significant (item C) and were approximately the same for the two treatments. Moreover the increment obtained was consistent for all varieties and for all cutting systems. To facilitate the solution of the problem posed, a table has been drawn up to show for each variety the yield under three different combinations of treatments as follows:

*Well managed, not fertilized* — mean of C<sub>2</sub>, C<sub>3</sub> under O

*Badly managed, not fertilized* — C<sub>4</sub>, under 0

*Badly managed, well fertilized* — C<sub>4</sub>, under mean of 2PK, M.

The comparisons are shown in Table 6. It may be seen that, because of mismanagement, the 1956 yield of DuPuits has been reduced from 2.8 to 1.7 tons per acre, a drop of 1.1 tons, and that by adding fertilizer the yield was brought back up to 2.4 tons from 1.7, an increase of 0.7. This provided a net deficit of 0.4 tons. For this variety fertilizer has not compensated for mismanagement. The same story holds true for Ranger. Vernal, however, presents a different picture. The loss suffered by that variety in 1956 through mismanagement was 0.4 tons per acre, i.e. a drop from 2.7 to 2.3 but the extra yield contributed to that mismanaged type by the addition of fertilizer was 1.0 tons, a net gain of 0.6 tons per acre. Grimm likewise was helped by fertilizers more than it was harmed by mismanagement.

The trend over the 3-year period 1955-7 followed precisely the pattern depicted in Table 6. DuPuits and Ranger suffered a net total loss of 0.4 and 0.1 tons per acre respectively whereas for Vernal and Grimm the extra yields were 1.6 and 1.7. These data show that only for the winter hardy varieties was there sufficient extra yield provided by fertilizers to compensate for faulty management. The corollary is that with DuPuits and Ranger management is the *sine qua non*.

### CONCLUSIONS

1. In this experiment Vernal was demonstrated to be superior to Ranger, DuPuits and Grimm under practically all conditions of cutting management and fertilizer applications investigated.

2. DuPuits was shown to be satisfactory for short-term stands that were properly managed.

3. The best harvesting practice for Ontario as suggested by this experiment is to cut two or three times a year. Cutting in September was detrimental to all varieties resulting in reduced stands and lowered yields in subsequent years. The harm done by mismanagement was greatest for non-hardy, wilt-susceptible types and least for winter-hardy, wilt-resistant types.

4. For Vernal (and by implication for similar strains of alfalfa) which, because of its winter hardiness and wilt resistance is able to withstand adverse conditions, the addition of moderate amounts of fertilizer was instrumental in overcoming to some extent the bad effects of poor management. For DuPuits (and presumably for other similar varieties such as

Alfa and Cardinal) stands of which may rather quickly be thinned out by winter killing and bacterial wilt, good management is of paramount importance and lack of it is not readily compensated for by the addition of fertilizers.

5. The addition of  $K_2O$  almost invariably resulted in more persistent stands and in higher yields. Its beneficial effect was most noticeable when the alfalfa was under stress caused by improper management and/or lack of resistance to an unfavourable environment. Potash x cutting treatments x hardiness interactions were highly significant in 1956 but not in 1957.

6. Reaction to  $P_2O_5$  varied markedly with the variety. Grimm profited greatly from additions of phosphorus whereas Ranger frequently suffered. All varieties benefited from phosphorus under the stress of harsh management but the hardy varieties and especially Grimm benefited much more than Ranger or DuPuits. The phosphate x cutting treatments x hardiness interactions were highly significant in 1956 but not in 1957. Improved stands caused by or concomitant with phosphate applications did not necessarily result in improved yields.

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# INFECTION OF WHEAT WITH *HELMINTHOSPORIUM SATIVUM* IN RELATION TO THE NITROGEN CONTENT OF THE PLANT TISSUES<sup>1</sup>

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## ABSTRACT

A modified detached leaf method was used in studying infection reactions in wheat inoculated with *Helminthosporium sativum*. The invasion and colonization of one-half of a leaf produced variable degrees of chlorosis in the uninvaded half. Nitrogen was retained and accumulated in the colonized portion of the leaf which also retained its green colour. Leaching readily removed nitrogen from the affected tissues. Inoculation of the culms resulted in increased production of low nitrogen kernels. The influence of *H. sativum* colonization in relation to nitrogen nutrition and rootrot injury is discussed.

## INTRODUCTION

Common rootrot of wheat caused by *Helminthosporium sativum* P. K. and B. may be classed as a debilitating disease. The plants become infected at any stage. However, this study is most concerned with the gradual involvement, as the season advances, of the crown, crown roots and adjacent tissues. Bolley (2), one of the first (1913) to recognize the importance of root diseases, described the conspicuous necrosis of the crown and crown root tissues of sickly wheat plants, the yellowing and death of leaves and tillers, all of which resulted in reduced yields. Similar descriptions of diseased plants were given by McKinney (6) in a comprehensive study of root troubles of wheat. He particularly mentioned the patchy condition of crops when rootrot caused by *H. sativum* was severe. Working in Australia, Hynes (4) described the symptoms of rootrot afflicted plants, mentioning their premature ripening and bleaching, reduced growth and severe necrosis of crowns, crown roots, and discoloured subcrown internodes.

The relationship between common rootrot infections and yields of cereals was studied by Greaney<sup>3</sup> in Manitoba. More recently, Sallans (7) found a close relationship between yields and disease in Saskatchewan as assessed by the intensity of lesions on the subcrown internode. In general, yields declined as disease increased.

Previous reports show, as in the contributions mentioned, that when rootrot is severe injury is readily assessed, but the ever-prevalent milder appearing infections need further consideration. The work reported here is an attempt to clarify the relationship between infection, nitrogen nutrition, and injury.

## METHODS

The detached leaf method with modifications was used extensively in the inoculation tests, mainly made on Thatcher wheat. Leaves from both seedlings and older plants growing in the greenhouse were inoculated.

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<sup>3</sup>Greaney, F. J. Losses caused by root diseases of cereals in Western Canada. *Unpublished data*. 1940.



Large leaves from older plants were cut into lengths that would fit in a Petri dish. Occasionally leaves from seedlings grown in culture tubes were employed; this technique, reported earlier (9), made it possible to obtain leaves from seedlings grown in water or various solutions. Inoculation was done by drawing the bottom half of the leaf over a sporulating culture of *H. sativum*. Both sides of the leaf were inoculated. The leaves were then arranged in a Petri dish bottom with the inoculated halves on moist filter paper. The inoculated half was usually covered, at least for 2 days, with moist filter paper. The uninoculated half protruded above the surrounding filter paper. Various solutions as well as water could be applied to the filter paper. The dishes were not covered but were kept under a large bell-jar to allow good aeration. When the tests were conducted in darkness the bell-jar was covered with a black cloth.

The degree of chlorosis, estimated visually, in the uninoculated upper half of the leaf was a good indicator of the extent of invasion in the inoculated lower half.

In addition to studies on detached leaves, leaves of seedlings growing in pots were enriched before inoculation by periodic application to the soil of a 3 per cent potassium nitrate solution. Similarly, applications through the period of kernel formation altered the nitrogen content of the grain and leaves from these grains could be subsequently tested by inoculation.

Leaf specimens were leached by washing them with two litres of water on filter paper in a funnel. Some specimens, especially fresh leaves, were soaked in water for about an hour before leaching. Total nitrogen determinations were made by the micro-Kjeldahl method.

## EXPERIMENTAL RESULTS

### *Nitrogen Content of Leaf Tissues in Relation to Infection*

In early work detached leaves were inoculated with *H. sativum* and floated on water and various solutions. In general, 3 per cent solutions of sucrose, dextrose and fructose supported an active invasion with subsequent distinct lesions, whereas similar solutions of potassium nitrate and asparagine retarded invasion and lesion appearance. Water alone allowed some growth and the development of small lesions. The results of numerous tests indicated that an ample supply of nitrogen reduced infection in the inoculated area and chlorosis in adjacent tissues.

In tests with detached inoculated leaves, those from wheat seedlings grown in sand, presumably low in nitrogen, were compared with leaves from seedlings grown in fertile soil. After 5 days the fungus was well established in the inoculated portion of the leaves. The leaves from seedlings grown in sand showed 100 per cent chlorosis of the uninoculated portion, whereas leaves from seedlings grown in soil showed 60 per cent.

In another test, young and old leaves from wheat plants in head were compared. Uninoculated check leaves were exposed to similar conditions to observe the natural fading with time. After 5 days the inoculated older leaves, low in nitrogen but uniformly green at the start, showed 85 per cent

chlorosis while the inoculated younger leaves, high in nitrogen, had 75 per cent. The results of a similar test are shown in Figure 1. In the checks chlorosis was 55 per cent in the older and only 5 per cent in the younger leaves.

In another set of tests the nitrogen content of the tissues was altered by application of potassium nitrate to seedlings grown in ordinary culture tubes (9). When the first leaf was fully expanded it was found that approximately 4 cc. of water had been used. Replacement was made by adding a 3 per cent potassium nitrate solution to some tubes and water, for checks, to others. Seedlings were allowed to grow for 3 days before leaves were taken for inoculation. After 3 days of incubation the results were as follows:

Specimens	Chlorosis
1. Leaves from seedlings grown in water. Inoculated.	80%
2. Leaves from seedlings grown in water. Not inoculated.	Faint colour loss
3. Leaves from seedlings grown in potassium nitrate. Inoculated.	5%
4. Leaves from seedlings grown in potassium nitrate. Not inoculated.	Uniformly green

In a similar test with Marquis wheat seedlings in pots in the greenhouse a 3 per cent potassium nitrate solution was added to the soil from time to time to enrich the leaves. When such leaves were inoculated in comparison with those from untreated plants, results similar to the above were obtained. The enriched leaves retained their green colour much better under infection than ordinary leaves.

The next experiment was designed to alter the nitrogen (protein) content of wheat seed so that leaves of seedlings grown from such seed could be tested by inoculation. Thatcher wheat plants growing in pots were fertilized during the period of kernel formation by periodic watering with a 3 per cent potassium nitrate solution. Comparable untreated plants served as checks. At harvest, piebald kernels, a clear indication of low protein (5), were commonly observed in the grain from the checks. The kernels from the fertilized plants were of good colour but slightly less plump than the check kernels. Seeds from each lot were germinated in tubes and the first leaves, when fully expanded, were taken for inoculation. After 4 days of incubation the leaves from the check seed showed 60 per cent chlorosis while those from the enriched seed had only 15 to 20 per cent. These results indicate that seed of high nitrogen content produces first leaves, at least, also high in nitrogen and that they are less injured by infections of *H. sativum* (Figure 2).

#### *Movement of Nitrogen to Infected Areas*

Several inoculation tests of detached wheat leaves were made to determine quantitatively the amount of nitrogen in the colonized green portion of the leaves and in the chlorotic upper uninoculated portion of the leaves at the conclusion of the incubation period. In addition, some leaching was done to determine how much soluble nitrogen may be lost through diffusion from the tissues into the surrounding moist filter paper. Large leaves from wheat plants at the heading stage were cut into 5-inch lengths for inoculation. Uninoculated leaves, as checks, were kept under similar conditions. The results in two representative tests after 5 days of incubation are shown in Table 1.

TABLE 1.—NITROGEN CONTENT OF DETACHED WHEAT LEAVES INOCULATED WITH *H. sativum* AND NON-INOCULATED

Specimens	Treatment	Total nitrogen, % (dry weight)		
		Test 1	Test 2	Average
Inoculated, colonized portion	Leached	3.40	3.57	3.48
Chlorotic, not colonized portion	Leached	2.07	2.08	2.07
Inoculated, colonized portion	Nil	4.40	3.75	4.08
Chlorotic, not colonized portion	Nil	3.53	2.66	3.09
Check, leaves not inoculated	Incubated	4.92		4.92
Check, leaves not inoculated	Soaked 5 hours, then leached and dried, not incubated		4.46	4.46
Check, leaves not inoculated	Dried, not incubated		4.41	4.41

To gather additional information on diffusable nitrogen, the amount in the surrounding filter paper at the conclusion of incubation was determined. The results showed for the colonized tissues a total N content of 2.74 per cent, for the chlorotic portion 2.25 per cent, and for the filter paper surrounding the colonized portion, about 2 per cent.

The data given for the above three experiments show a higher nitrogen content in the colonized tissue and a loss of nitrogen through leaching. Although it was not measured separately a fair amount of fungus growth extended away from the colonized tissues into the surrounding paper and a certain amount of nitrogen would be utilized in this outside thallus. The leaching demonstrated a marked loss of nitrogen from both the colonized and bleached tissues, especially the latter.

Barley leaves were used in one inoculation test with the following results:

Specimens	Total nitrogen (dry weight) %
Check, uninoculated pieces of the leaves dried at room temperature	6.04
Inoculated colonized portion of leaves	5.80
Chlorotic uninoculated portions	4.94

The leaves of barley reacted similarly to those of wheat. The chlorotic portion of the specimens lost nitrogen, some of which, no doubt, was drawn into the invaded colonized zone of the leaf.

#### *Relationships between Infection and Nitrogen Content of Wheat Kernels*

Since experimentally-produced wheat seed of high nitrogen content gave first leaves that were high in nitrogen, a study was made of piebald kernels naturally low in protein. Several tests were conducted using piebald and normal kernels selected from the same sample. The seeds were germinated in tubes and the first leaves were used for inoculation. The results with kernels selected from a sample of Garnet wheat showed 100 per cent chlorosis of the leaves from piebald kernels with only 5 per cent chlorosis of the leaves from normal kernels.

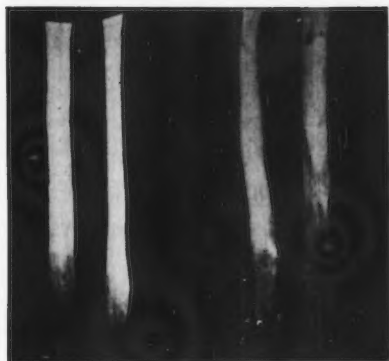


FIGURE 1. Leaves of Marquis wheat taken from greenhouse grown plants at heading stage and inoculated on lower portions with *H. sativum*. Chlorosis was greater in the uninoculated portions of lower leaves (*left*) than of upper leaves (*right*).



FIGURE 2. Seedling leaves of Red Bobs wheat inoculated on lower portions with *H. sativum*. The covering filter paper has been removed to show the colonization. Leaves on the *left* were from piebald kernels and show greater chlorosis than those on the *right* from normal kernels.



Not all tests were as distinctive as the one with Garnet wheat but all did show the same tendency for a better retention of chlorophyll in the tissues of higher nitrogen content. Tests were conducted with samples of Thatcher, Regent, Red Bobs, Marquis, Mindum and Pelissier wheats.

A logical next step appeared to be to explore the influence of severe infections in the adult plant on the quality of the kernels. This approach was made on the assumption that as in the leaf inoculations, nitrogen would be drawn to the infection site. Thus, if there was a sufficient diversion of nitrogen from the translocatory stream, the protein content of the grain would be reduced.

In one series of trials, wheat plants growing in a large bed in the greenhouse were inoculated at flowering time. The inoculum, consisting of a heavy suspension of spores in a little mineral oil, was inserted into the culm with a needle. The inoculation points were at the base of the peduncle or in the internode below. A good lesion developed. A second series of inoculations was made by the same method on plants growing in crocks. At harvest time, heads from diseased culms and similar clean culms were threshed and the kernels examined with results as follows:

	First Series			Second Series		
	Number of kernels			Number of kernels		
	Total	Piebald		Total	Piebald	
		No.	%		No.	%
Inoculated culms	232	19	8	244	126	51
Clean culms	132	0	0	138	28	20

These data indicate that inoculation of adult plants results in an increased production of low quality kernels.

#### DISCUSSION

These experiments show that detached wheat leaves, when inoculated with *H. sativum*, are rapidly invaded and colonized. The invaded zone tends to retain its green colour while the adjacent zone becomes chlorotic. It is thought that the colonized tissues, where the fungus is active, function as a physiological "sink" in the sense of Maskel and Mason, as discussed for wheat by Van de Sande-Bakhuyzen (11). Marked reactions occur in plant tissues when invaded by fungi, especially changes in the permeability of cell membranes, a phenomenon explored by Thatcher (10) with convincing results. Infection of wheat leaves by *Erysiphe graminis tritici*, studied by Allen (1) is of particular interest because of the tendency for nutrients to accumulate at the infection site. Moreover, his observation of the green island phenomenon wherein the chlorophyll is destroyed and subsequently reforms or reappears is analogous to the retention of chlorophyll, with often an appearance of greater density, in tissues colonized by *H. sativum*. The intensified green colour appears even in the early stages of infection. It is

suspected that the nitrogen drawn to the infection site may in some way protect the chlorophyll, thus acting like an inflammation in injuries to animal tissues. Shaw and Samborski (8), using radioactive tracer techniques, tested many parasite-host combinations, including a *Helminthosporium* sp. (probably *H. sativum*), which produced an accumulation of substances in the infection locus. There are many reports, based chiefly on field material, showing more protein, hence nitrogen, in rusted tissues than in non-rusted tissues. Greaney, Woodward and Whiteside (3) in a comprehensive study of rusted wheat established this trend, revealing an accumulation of protein in the vegetative tissues along with a decrease in the grain.

In interpreting the present results there are two main points of pathological interest—first, the accumulation of nitrogen at the infection site and, second, the loss of nitrogen by diffusion or leaching. In both instances the host is deprived to a variable extent, depending upon conditions, of its normal supply of this essential element. Extensive invasion and colonization is characteristic of *H. sativum* in causing rootrot. An active colonization at the crown, including crown roots, is well located to intercept nitrogen from its only source, the soil. If interception does take place and some of the nitrogen is diverted to the fungus colony, a reduction in growth and quality of the grain seems inevitable. Especially might protein be reduced when the invasion is active during the period of kernel formation as indicated in this study. In addition, the quality of the protein might be affected. The great variations in protein content and quality between samples of grain grown in the same field or smaller areas is well known (5). Some of this variation may be caused by infections of rootrot fungi.

In further consideration of quality a comparison of rust and rootrot etiology is of interest. With rust the invasion is confined to the photosynthetic tissues, the carbohydrate source, and hence the weight of grain is reduced; with rootrot, the nitrogen source (and in turn the protein content) is believed to be affected primarily.

#### ACKNOWLEDGEMENTS

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# FERTILIZERS AND THE NUTRITIVE VALUE OF WHEAT GROWN ON A SULPHUR-DEFICIENT GREY WOODED SOIL<sup>1</sup>

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## ABSTRACT

Rate of gain, efficiency of food utilization, and nitrogen retention by weanling rats were used as criteria to determine the nutritive value of wheat grown on a sulphur-deficient Grey Wooded soil. All diets were supplemented with minerals, vitamins, and lysine. Grain from an area continuously cropped to wheat-fallow and from an area cropped to a 5-year grain-legume rotation were compared. On Breton loam, a Grey Wooded soil, grain is highly responsive to fertilization with nitrogen; and legumes are very highly responsive to sulphur fertilization. Samples were fed from plots receiving the same fertilizer treatments in each cropping system. Fertilizer treatments and cropping systems both caused occasional significant differences, and were associated with some consistent trends for differences, in nutritive value of wheat. In general, the grain was of substantially superior nutritive value when grown following legumes; in one year these differences tended to be greater with fertilizer treatments which increased yields. During the same year fertilization with manure tended to improve the nutritive value of fallow wheat. The animal growth and food efficiency were closely related to the protein content of the foods. There were differences between results obtained in 2 successive years. While the differences may have been due to the effect of seasons or to the biennial application of fertilizers they appeared to be closely related to protein content of the grain, a characteristic which long-time data have shown to be highly variable in grain from the Breton plots.

## INTRODUCTION

Cropping practices which include rotations and fertilizer treatments have been shown to improve yield and to affect composition of wheat grown on a sulphur-deficient Grey Wooded soil (12). The yield and protein content of hays grown on that soil have also been increased by fertilization. As measured by feeding trials with rabbits the feed value of legume hays from plots receiving sulphur fertilization was higher than that of hay from adjacent unfertilized plots (5). Since there are in Alberta millions of acres of sulphur-deficient soil, generally similar to that at the Breton Plots, it is important to know whether the nutritive value of wheat grown on those plots is affected by soil management practices. The purpose of this investigation was to determine whether these practices are causing differences in nutritional value of wheat as measured by rate of gain, efficiency of food utilization, energy and nitrogen digestibility and nitrogen retention in the rat.

Several investigators (11, 15, 18) have used the rat as an experimental animal to determine the nutritive value of grains. Rats should be particularly useful for evaluating grains as swine food as these two species have rather similar amino acid requirements (8).

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Rosenberg *et al.* (15) and Sure (18) both established that lysine was low in wheat and was a limiting factor for rat growth. In earlier work Mitchell *et al.* (11) found that, if lysine was supplemented in sufficient quantities, tryptophan appeared as the next limiting amino acid in wheat. Rosenberg *et al.* (16) later showed that a diet of wheat supplemented with 0.8 per cent lysine produced rat gains equal to those obtained with a stock diet well balanced with the essential amino acids. Hutchinson *et al.* (6, 7), also working with rats, found lysine to be the most limiting amino acid in white bread. They reported that supplementation with lysine resulted in marked improvement in animal growth.

The nutritive importance of both quantity and quality of protein in wheat has been demonstrated. McElroy *et al.* (10), using Alberta grains from different soil zones, found that rats fed wheat containing 17.3 per cent protein gained 70 per cent more rapidly than rats fed wheat containing 8.9 per cent protein. It was shown later that these results were applicable to swine (9). Also using rats, Bender (4) found that fortification of bread protein with certain amino acids increased net protein utilization by over 70 per cent. He listed lysine, threonine and methionine, in that order, for limiting effects on the nutritive value of bread for rats.

The quality of wheat protein has been found to vary with fertilization and crop rotations. Renner *et al.* (14) studied the effect of various fertilizers and two crop rotations (wheat after legumes and wheat after fallow) on the amino acid content of grain grown on the Breton Plots. They found that, even though there was an increase in the quantity of protein in wheat after legumes, there was sometimes a decrease in quality as measured by the proportion of nine essential amino acids. However, when fertilizer containing sulphur was applied to grain grown in rotation with legumes, the essential amino acids were increased. This suggests that fertilized wheat grown at Breton on sulphur-deficient plots in rotation with legumes may have an increase in the proportion of certain amino acids which may affect its nutritive value.

TABLE 1.—FERTILIZER TREATMENTS FOR SELECTED PLOTS AT BRETON

Plot No.	Approx. pH	Symbol used	Treatment	Approximate nutrient applications <sup>1</sup> lb./ac./yr.			
				N	P	K	S
1 & 5	6.2	Check	None	—	—	—	—
2	6.1	M	Manure, 20 tons, once in 5 years	32 <sup>2</sup>	20 <sup>2</sup>	25 <sup>2</sup>	15 <sup>2</sup>
3	5.8	NPKS	Ammonium phosphate 16-20-0 + potassium sulphate 0-0-50	8	10	15	7
4	5.8	NS	Ammonium sulphate 21-0-0	8	—	—	7
6	7.1	Lime		—	—	—	—
9	6.2	NPS + M	Ammonium phosphate 16-20-0 + manure as plot 2	40 <sup>2</sup>	30 <sup>2</sup>	40 <sup>2</sup>	15 <sup>2</sup>

<sup>1</sup>These are calculated averages. For convenience fertilizers are applied every other year. Applications were made in 1956 but not in 1957. (Exceptions: manure on fallow wheat plots 1957 and on rotation plots 1955).

<sup>2</sup>An average nutrient content was used for manure.

## MATERIALS AND METHODS

*Breton Plot Wheat*

A description of the Breton Plots and a full account of the results for the various cropping and fertilizer treatments is available (12). The application of sulphur to these plots has increased yield of legume hay about five times. Yields of cereals following the fertilized legume are approximately doubled.

This investigation was concerned with wheat from six plots established in 1930 on each of two cropping systems—wheat on fallow; and a 5-year rotation consisting of wheat, oats, barley and 2 years of legume hay. Table 1 lists the fertilizer treatments and provides related information for the six plots.

Since the rations used in these experiments were fortified with a mineral mixture, details regarding the mineral composition of the grains are not included in this report. Analyses of the grains revealed normal mineral compositions with approximately 0.4–0.5 per cent P; 0.5 per cent K; 0.10–0.15 per cent S; 0.02–0.04 per cent Ca; 0.10–0.15 per cent Mg and 0.01 per cent Na in the 1956 grains. In the 1957 grains P, K and S were somewhat lower, being about 0.38, 0.34 and 0.10 per cent respectively. Variations were generally rather slight and did not show any consistent relationship to fertilizer treatments.

*Analytical Procedures*

Wheat for the feeding trials was harvested about September 1. After cleaning to remove weed seeds and all other extraneous matter the grain was ground in a Wiley mill to pass a 20-mesh screen. For Experiment 5 each composite sample consisted of equal weights of grain for the 2 or 3 years concerned.

Total nitrogen content of feeds, feces and urine was determined by the Kjeldahl-Gunning method (13) using mercury as catalyst. The energy content of the food and feces was determined using a Parr Oxygen Bomb Calorimeter (1).

*Rat Feeding Experiments*

Complete mineral and vitamin supplements as used by Sibbald *et al.* (17) were added to all diets. L-lysine was added at a level of 0.9 per cent which was 0.1 percentage units higher than the level suggested by Rosenberg *et al.* (16). This addition was made to all diets so that lysine, which has been found to be low in wheat, would no longer be limiting. Any differences obtained from feeding wheat grown on different plots should, therefore, be the result of factors other than lysine, minerals or vitamins.

Male albino weanling rats of the Sprague-Dawley strain were used in this study. They were allotted to individual cages and fed assigned foods during a preliminary acclimatization period of 1 week. The animals were then transferred to metabolism cages for a week, during which time weight gain and food consumption were recorded and feces and urine were collected. Data reported are for the 7-day metabolism period only. The feeding procedures and methods of fecal and urine collection were similar to those reported by Sibbald *et al.* (17). Analysis of variance was conducted on the rat data.

The following formulae were used in the derivation of some of the figures in the tables. (It should be noted that digestibility figures are for apparent rather than true digestibility).

$$(a) \text{ Digestible Energy}(\%) = \frac{\text{Gross food energy} - \text{Fecal energy}}{\text{Gross food energy}} \times 100$$

$$(b) \text{ Digestible Nitrogen}(\%) = \frac{\text{Gross food nitrogen} - \text{Fecal nitrogen}}{\text{Gross food nitrogen}} \times 100$$

$$(c) \text{ Nitrogen Retained}(\%) = \frac{\text{Food N.} - (\text{Fecal N.} + \text{urinary N.})}{\text{Food nitrogen}} \times 100$$

### RESULTS AND DISCUSSION

Three feeding trials were conducted using wheat grown in 1956. Data for those experiments are reported in Tables 2 and 3.

Although there are few statistically significant differences between the data shown in Table 2 the data show definite trends. Rats fed wheat from plots receiving fertilizers supplying sulphur (including manure) grew faster and made more efficient gains than animals fed grain from the unfertilized and lime plots. Since there are neither statistically significant differences nor any discernable trend of differences for digestible energy, digestible nitrogen, nitrogen retention and digestible energy per gram of digestible nitrogen, these data do not appear to be helpful in explaining the growth and food efficiency differences in this experiment. These results are similar to those of Bains (2, 3) who found an increase in weight gains of rats fed wheat which was grown after a green manure although there was not a corresponding increase in digestibility of the food.

Experiment 2 (Table 3) included four treatments of wheat grown after legumes, which had been tested previously in Experiment 1 and a comparison with those results is interesting. The NPKS treatment, one of the two significant differences in Experiment 2, did not show a significant difference in Experiment 1. Moreover, animal growth and food efficiency for the NPS + M treatment were no better than for the check in the

TABLE 2.—EFFECT OF WHEAT GROWN AFTER LEGUMES ON RAT GROWTH AND FOOD EFFICIENCY (EXPERIMENT 1—1956)

Treatment	Yield bu./ac.	N in grain %	Average <sup>1</sup> rat gain /7 days gm.	Av. food consumed /7 days gm.	Av. food /gm. gain gm.	Digestible <sup>2</sup>		Nitrogen <sup>2</sup> retained %	Digestible <sup>2</sup> energy/gm. dig. N Cal.
						Energy %	Nitrogen %		
Check	13.8	2.12	13.8	52.6	3.8	87.0	86.6	40.2	179
L	13.4	2.46	12.9	50.0	3.9	86.3	88.6	35.0	150
M	34.8	2.41	17.8	64.1	3.6	86.8	87.0	44.2	157
NS	38.7	2.37	21.5*	55.9	2.6*	84.6	83.3	37.1	164
NPKS	33.6	2.34	17.1	57.8	3.4	88.4	86.7	42.2	170
NPS + M	42.3	2.32	20.4*	62.9	3.1	85.5	84.2	37.7	168
*L.S.D.	—	—	5.1	—	0.9	—	—	—	—

<sup>1</sup>Rat data average of 4 replicates

<sup>2</sup>Digestibility data on oven-dry weight of food consumed

TABLE 3.—EFFECT OF WHEAT GROWN AFTER FALLOW AND OF WHEAT GROWN AFTER LEGUMES ON RAT GROWTH AND FOOD EFFICIENCY. (EXPERIMENTS 2 AND 3—1956)

Treatment	Yield bu./ac.	N in grain %	Average <sup>1</sup> rat gain /7 days gm.	Av. food consumed /7 days gm.	Av. food /gm. gain gm.	Digestible <sup>2</sup>		Nitrogen <sup>3</sup> retained %	Digestible <sup>2</sup> energy/gm. dig. N Cal.
						Energy %	Nitrogen %		
EXPERIMENT 2									
Wheat after Legumes									
Check	13.8	2.12	12.0	49.2	4.1	86.1	82.8	28.6	188
NS	38.7	2.37	16.1*	57.5	3.6	86.4	85.5	36.3	163
NPKS	33.6	2.34	19.4*	63.2	3.2	85.4	81.3	36.6	155
NPS + M	42.3	2.32	11.6	49.6	4.3	86.9	84.2	31.9	171
Wheat after Fallow									
Check	8.6	1.97	10.7	48.1	4.5	84.3	80.5	30.2	202
NS	7.1	1.99	8.6	46.4	5.4	84.3	81.8	30.7	198
NPKS	10.3	1.88	7.6	43.9	5.8	85.8	80.2	28.9	220
NPS + M	14.5	2.12	14.0	52.8	3.8	87.1	85.7	35.6	182
*L.S.D.	—	—	7.0	—	—	—	—	—	—
EXPERIMENT 3									
Wheat after Legumes									
Check	13.8	2.12	11.4	51.5	4.5	85.8	85.1	27.6	181
M	34.8	2.41	14.7	59.6	4.0	86.0	84.8	34.5	159
NS	38.7	2.37	16.7	61.1	3.6	85.1	83.8	37.1*	164
NPS + M	42.3	2.32	19.3	67.1	3.5	84.2	81.9	34.2	171
Wheat after Fallow									
Check	8.6	1.97	8.0	50.0	6.2	84.0	80.1	29.2	204
L	10.8	2.11	11.2	55.2	4.9	84.0	79.1	36.1*	204
M	8.3	2.10	15.6	60.4	3.9	86.2	85.2	37.1*	186
NS	7.1	1.99	7.7	47.5	6.2	84.6	80.9	32.9	201
*L.S.D.	—	—	—	—	—	—	—	7.5	—

<sup>1</sup>Rat data average of 4 replicates in both experiments<sup>2</sup>Digestibility data on oven-dry weight of food consumed

second trial although there had previously been a significant difference. The NPS + M treatment was, therefore, included in Experiment 3 where results comparable to those in the first experiment were obtained. In other respects data for wheat after legumes in the second trial are very similar to those in the first experiment.

In Experiment 2 there were important differences in food efficiency and animal gains when wheat after legumes and wheat after fallow are compared. The only fertilizer treatment which appears to have improved the nutritive value of wheat after fallow is NPS + M. As the grain receiving the NPS + M treatment was higher in protein a superior nutritive value was to be expected (10). Digestible energy per gram of digestible nitrogen may be related to the nitrogen content of the grain and to animal gains and food efficiency but other relationships for digestible values are doubtful.

The data for food efficiency and animal gains in Experiment 3 (Table 2) are in rather good agreement with those of the second experiment when the anomalous data for NPS + M are disregarded. In Experiment 3, for wheat after fallow, the M treatment appears to have had favourable effects while it is more doubtful if the lime was beneficial. Nitrogen retention values are significantly different for several treatments in Experiment 3 although there were rather inconsistent results for that determination in the first two experiments.



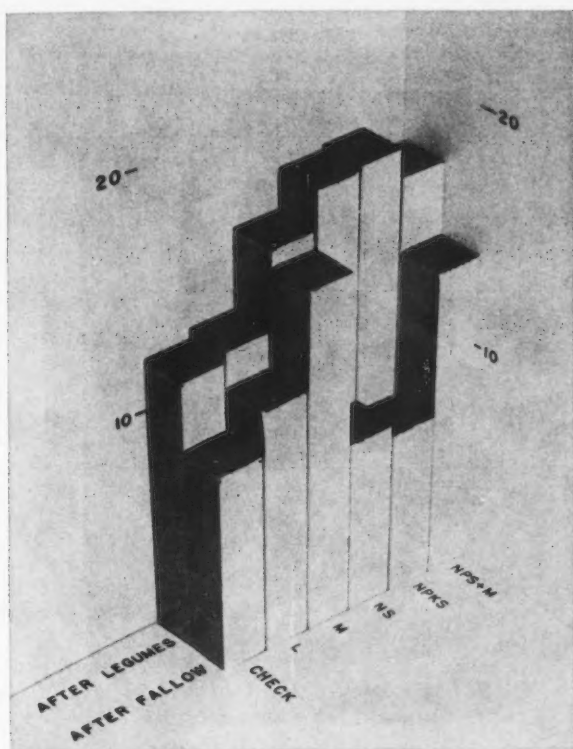


FIGURE 1. Average rat gains in grams per week. 1956 Breton wheat.

Figure 1 is a diagrammatic presentation of feeding trial results obtained in Experiments 1, 2 and 3 with 1956 wheat.

The yield data for 1956 reported in Tables 2 and 3 merit special mention. Yields for wheat after legumes are in excellent agreement with long-time results, while yields for wheat after fallow are very sharply lower than corresponding long-time data. The 1956 wheat after fallow yields are especially low for plots which received the treatments M, NS, NPKS and NPS + M where yields are less than one-half of the long-time averages.

Yield data for 1957, presented in Table 4, agree well with long-time averages with one exception. Yields of wheat after fallow for plots which received manure are somewhat high. However, this is readily explained by the fact that this was the first crop following an application of manure.

Data for animal gains and food efficiency for wheat grown in 1957 are only partly in agreement with the 1956 results. The various fertilizer treatments appear to have had little if any effect on the nutritive values of the 1957 grain—a result distinctly different to that obtained with 1956 grain. However, in 1957 wheat grown after legumes appears to be more



TABLE 4.—EFFECT OF WHEAT GROWN AFTER FALLOW AND OF WHEAT GROWN AFTER LEGUMES ON RAT GROWTH AND FOOD EFFICIENCY. (EXPERIMENT 4—1957)

Treatment	Yield bu./ac.	N in grain %	Average <sup>1</sup> rat gain /7 days gm.	Av. food consumed /7 days gm.	Av. food /gm. gain gm.	Digestible <sup>2</sup>		Nitrogen <sup>2</sup> retained %	Digestible <sup>2</sup> energy/gm. dig. N Cal.
						Energy %	Nitrogen %		
<i>Wheat after Legumes</i>									
Check	11.6	2.82	21.9	62.6	2.9	86.4	87.5	41.5	132
L	12.3	2.84	18.4	58.2	3.2	86.4	88.6	41.0	131
M	21.2	2.68	21.4	64.9	3.0	87.3	88.1	44.2	140
NS	36.5	2.72	18.6	57.6	3.1	83.9	82.7	41.5	143
NPKS	38.5	2.42	18.2	55.4	3.0	85.2	82.7	42.9	162
NPS + M	30.0	2.82	23.7	63.1	2.7	85.7	85.8	46.8	134
<i>Wheat after Fallow</i>									
Check	17.0	2.03	13.6	50.9	3.7	83.3	75.2	35.2	203
L	17.8	2.00	16.1	51.4	3.2	84.0	78.8	40.3*	200
M	43.8	2.26	15.3	53.2	3.5	86.5	84.8	48.4*	168
NS	25.8	2.12	15.2	53.3	3.5	84.6	78.4	41.1*	188
NPKS	33.9	2.03	11.1	45.9	4.1	85.7	80.1	40.2*	196
NPS + M	40.7	2.35	17.5	54.1	3.1	86.4	83.8	44.0*	167
*L.S.D.	—	—	—	—	—	—	—	4.0	—

<sup>1</sup>Rat data average of 6 replicates<sup>2</sup>Digestibility data on oven-dry weight of food consumed

nutritious than wheat grown after fallow, as determined by animal gains and food efficiency. This result is in agreement with the 1956 findings. The differences and similarities between feeding trial results for the 2 years may be partly explainable on the basis of grain nitrogen content. In 1956, plot treatments caused appreciable differences in protein content of wheat after legumes but this was not the case in 1957. Data for wheat after fallow show about the same range in protein content, rate of animal gains and food efficiency for the 2 years. For both years, the more favourable rates of gain and food efficiencies were obtained with grain of higher protein content. This result is in accord with the data of McElroy *et al.* (10).

The foregoing experiments did not definitely establish the cause or causes of nutritive differences found between 1956 grains from variously fertilized plots. Fertilization caused differences in protein content of the grain and the nutritive differences found may be due only to these variations in amount of protein. Although plot treatments have resulted in some slight differences in mean protein content of the wheat produced under a given crop sequence at Breton (12, 14) there has not been a consistent effect. Table 5 illustrates the variability which has occurred. The rather uniform protein content of 1957 wheat following legumes compares more closely with the average results than does the 1956 data. Baking results with Breton wheat show large variations from year to year (12) and those results support the probability of similar variations in the effects of fertilizer treatments on nutritive values.

Perhaps the 1956 differences occurred because fertilizer was applied that year, whereas the 1957 crop was dependent on residual effects of fertilizers. To test this possibility a fifth feeding experiment was conducted using composite samples. The data are given in Table 6. Those results show that, for the composite samples used, fertilizer treatments within the two

TABLE 5.—COMPARISON OF NITROGEN CONTENT OF CHECK PLOT AND OTHER BRETON WHEATS. ALL ANALYSES 1931–1957

	Treatments					
	Check	M	NPKS	NS	L	NPS + M
<i>Rotation wheat</i>						
N per cent, mean of 31 analyses	2.39	2.25	2.29	2.46	2.41	2.36
	<i>Number of times</i>					
N per cent, $\pm 0.1$ of check	—	10	8	5	11	12
N per cent, more than 0.1 below check	—	18	16	9	11	8
N per cent, more than 0.1 above check	—	3	7	17	9	11
<i>Wheat on fallow</i>						
N per cent, mean of 14 analyses	2.04	2.08	1.93	1.98	2.09	2.18
	<i>Number of times</i>					
N per cent, $\pm 0.1$ of check	—	6	8	6	7	6
N per cent, more than 0.1 below check	—	2	4	7	2	7
N per cent, more than 0.1 above check	—	6	2	1	5	1

TABLE 6.—RAT GROWTH AND FOOD EFFICIENCY FOR COMPOSITE WHEAT SAMPLES FROM FERTILIZED AND UNFERTILIZED YEARS. (EXPERIMENT 5)

Treatment	N in grain %	Average <sup>1</sup> rat gain /7 days gm.	Av. food consumed /7 days gm.	Av. food /gm. gain gm.	Digestible <sup>2</sup>		Nitrogen <sup>2</sup> retained %	Digestible <sup>2</sup> energy/gm. Dig. N Cal.
					Energy %	Nitrogen %		
<i>Fertilized Years—1950, 1952—Composite<sup>3</sup></i>								
Check	2.56	22.7	60.8	2.7	85.7	85.2	41.2	156
NPS + M	2.50	23.7	64.1	2.8	86.3	87.4	45.6	156
<i>Unfertilized Years—1947, 1949, 1955—Composite<sup>3</sup></i>								
Check	2.76	27.1	65.8	2.5	86.4	87.0	40.3	147
NPS + M	2.84	26.3	62.6	2.4	85.8	86.6	44.5	143

<sup>1</sup>Rat data average of 6 replicates<sup>2</sup>Digestibility data based on oven-dry food consumption<sup>3</sup>Equal portions by weight were used in making composites

sets of samples had not affected nutritive values as measured by rat growth and food efficiency. However, there was a strong trend for a difference between sets of samples, the grain of higher protein content again giving better animal performance.

Fertilization may have caused changes in the proportions of some essential amino acids (14) and such differences, if existent, may have contributed to the variations in food value. It is possible that lysine supplementation of the diets in these experiments has masked differences which might have been revealed if that amino acid had not been added, as improvement in rat growth resulting from addition of lysine to wheat flour or grain has been reported frequently (6, 7, 15, 16, 17). However Renner *et al.* (14)

found the proportion of lysine in Breton wheat to be little affected by fertilization or cropping system although fertilizer treatments apparently had an effect on the lysine proportions in barley grown as the first crop after legumes. The possibility of the lysine supplementation altering a nutritive difference involving that amino acid in these experiments is therefore doubtful.

These experiments have shown that crop rotation and fertilization at the Breton Plots have affected the nutritive value of the wheat produced. The results show that improvements in food value of the grain are closely related to changes in its protein content. Amino acid assays were not performed to determine whether amino acid proportions differ in the grains which were used. For that reason the possible nutritive significance of such changes has not been clarified by these experiments and further investigations are therefore contemplated.

The determinations of digestible energy, digestible nitrogen, nitrogen retention and digestible energy per unit of digestible nitrogen have been of doubtful usefulness in these experiments. For that reason the determination of animal gains and food efficiency, supplemented by amino acid assays of the grain, would appear to be of more value for further studies of this kind.

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# CONTROL OF THE ONION MAGGOT, *HYLEMYA ANTIQUA* (MEIG.) (DIPTERA: ANTHOMYIIDAE), WITH INSECTICIDES IN ORGANIC SOILS OF SOUTHWESTERN QUEBEC

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## ABSTRACT

In organic soils of southwestern Quebec from 1955 to 1958, dieldrin heptachlor and endrin wettable powders mixed at the rate of 1 ounce of toxicant per pound of onion seed applied for the control of the onion maggot, *Hylemya antiqua* (Meig.) were highly effective. The heptachlor treatment appeared to stimulate plant growth. Toxaphene as seed treatment was poor, while di-syston also as seed treatment was effective but reduced germination by one-third. A soil surface treatment with chlordane dust at 4.5 pounds of toxicant per acre gave also a fair control where seed had not been treated. Combinations of dieldrin or DDT seed treatments with chlordane or aldrin soil surface treatments when plants averaged 2 inches high were not more effective than a seed treatment alone.

## INTRODUCTION

The main chemicals recommended for many years against the onion maggot, *Hylemya antiqua* (Meig.), in Canada were mercuric chloride oil-bordeaux emulsion and mercurous chloride (calomel) (1). Calomel seed treatment was the standard recommendation in the province of Quebec until replaced by DDT in 1952. Chlordane dust as soil surface applications was also found to be effective in the mineral soils of Quebec in experiments carried out from 1948-1951 (4) and from 1951-1954 (5). Effective control was obtained in British Columbia in 1953 by treating the seed with DDT and dieldrin wettable powders (2). In 1952, seed treatment with DDT and soil surface treatment with aldrin<sup>2</sup> were also effective in mineral soils of Quebec.

This is a report on methods and rates of application of some chlorinated hydrocarbon insecticides and their value in the control of the onion maggot in muck soils.

## MATERIALS AND METHODS

The plot design was similar to that described by Perron *et al.* (5). Seeding was done with a Planet Jr. seeder No. 4 at the heavy rate of approximately 1/4 of an ounce per 120 feet of row to produce a thick stand of onions which facilitated the movement of larvae from plant to plant, and increased the number of plants killed (7). Onion (*Allium cepa* L.) of the variety Early Yellow Globe Danvers was used throughout. The following insecticides were tested: dieldrin (1, 2, 3, 4, 10, 10-hexachloro-*exo*-6,7-epoxy-1, 4, 4a, 5, 6, 7, 8, 8a-octahydro-1, 4-*endo*, *exo*-5, 8-dimethanonaphthalene), endrin (1, 2, 3, 4, 10, 10-hexachloro-*exo*-6, 7-epoxy-1, 4, 4a, 5, 6, 7, 8, 8a-octahydro-1, 4-*endo*, *endo*-5, 8-dimethanonaphthalene), heptachlor (1 (or 3a),

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<sup>2</sup> Perron, J. P. Unpublished report.

4, 5, 6, 7, 8, 8-heptachloro-3a, 4, 7, 7a-tetrahydro-4, 7-methanoindene), DDT (1, 1, 1-trichloro-2, 2-bis (*p*-chlorophenyl) ethane), toxaphene (chlorinated camphene containing 67-69% chlorine), and di-syston (*O*, *O*-diethyl *s*-2-(ethylthio) ethyl phosphoro-dithioate) wettable powders as seed treatments; aldrin (1, 2, 3, 4, 10, 10-hexachloro-1, 4, 4a, 5, 8, 8a-hexahydro-1, 4-*endo*, *exo*-5, 8-dimethanonaphthalene) and heptachlor emulsible concentrates as soil and drench treatments; chlordane (1, 2, 4, 5, 6, 7, 8, 8-octachloro-3a, 4, 7, 7a-tetrahydro-4, 7-methanoindan) and aldrin dusts as soil surface treatments. The insecticides and rates used are shown in Table I. The four following methods of application were used:

*Seed Treatment:* The seed was moistened with water, mixed with a wettable powder of the insecticide until the chemical adhered uniformly to its surface (3), and sown immediately.

*Soil Treatment:* The insecticide was sprayed on the soil surface with a small compressed-air sprayer, then worked into the top 2 or 3 inches of soil with a rake, and the seed was sown immediately.

*Soil Surface Treatment:* The insecticide was applied at the bases of the seedlings by shaking the dust through a small cheesecloth bag on three dates at 7- to 10-day intervals beginning on May 25 (5).

*Drench Treatment:* The insecticide was applied along the row of onions about June 1 by soaking the soil 1 inch on each side of the row to a depth of approximately 2 inches. The application was made with a watering-can fitted with a long metal spout.

To promote a severe infestation, several thousand adults were liberated over the plots each year (6). Maggot damage was appraised three times a week from the time the first injury was observed, in late May or early June, until the first week of July; thereafter, once a week until harvest. In 1955, 1956 and 1958, the infested plants were pulled and left on the soil, to promote infestation of the remaining plants. In 1957 the infested plants were clipped with scissors at ground level so as not to displace the maggots and favour migration to other plants. The latter method did not increase infestation and was discontinued. Yield records were taken at harvest each year and percentage of damaged bulbs was calculated in 1958 only. The percentage mortality was calculated on the total plants remaining, plus those that had been killed.

## RESULTS AND CONCLUSIONS

All treatments were significantly more effective than the checks (Table I) and seed treatments with dieldrin, heptachlor and endrin were significantly better than all other treatments, giving respectively an average per cent plants killed in the angular values of 0.8 for 4 years, 0.4 for 2 years and 1.2 for 1 year, compared to 5.2 in the checks for the 4 years. These three seed treatments were the most economical and practical, and there was no significant difference between dieldrin and heptachlor but the latter appeared to stimulate plant growth. Seed treatments with DDT and toxaphene did not give sufficient protection to the crop, the latter treatment being also toxic to the seedlings. Di-syston gave good control of the insects but was very toxic to seedlings, reducing the stand of onions by more



TABLE 1.—RATES OF INSECTICIDE APPLICATIONS, PER CENT ONION PLANTS KILLED (1955-58), IN ANGULAR VALUES,<sup>1</sup> PER CENT GAIN IN YIELD AND INJURED BULBS IN MUCK SOIL PLOTS FOR THE CONTROL OF THE ONION MAGGOT, STE. CLOTHILDE, QUE.

Insecticide <sup>a</sup> and method of application	Toxicant per pound of seed or per acre	Plants killed <sup>a</sup>				Per cent	
		1955	1956	1957	1958	Gain in yield over check	Injured bulbs at harvest (1958)
<i>Seed treatment</i>							
Heptachlor W. P.	1 oz.	—	—	0.4	0.3	66.1	16
Endrin W. P.	1 oz.	—	—	—	1.2	47.2	8
Dieldrin W. P.	1 oz.	0.3	1.0	1.3	0.5	55.7	12
Toxaphene W. P.	1 oz.	—	6.0	—	—	0.0	—
Di-syston W. P.	1 oz.	—	—	0.7	—	46.3	—
DDT W. P.	2 oz.	1.3	4.8	—	—	52.5	—
<i>Soil surface</i>							
Aldrin dust	2.5 lb.	1.5	3.5	—	—	43.2	—
Chlordane dust	2.5 lb.	0.8	3.8	—	2.2	45.7	8
Chlordane dust	4.5 lb.	—	—	—	1.3	64.3	8
<i>Seed and soil surface (one application each)</i>							
{ Dieldrin (Seed)—one appl.	1 oz.	0.4	—	—	—	60.2	—
{ Chlordane (Soil surface)	2.5 lb.						
{ Dieldrin (Seed)—one appl.	1 oz.	0.3	—	—	—	61.2	—
{ Aldrin (Soil surface)	2.5 lb.						
{ DDT (Seed)—one appl.	2 oz.	1.1	—	—	—	57.3	—
{ Aldrin (Soil surface)	2.5 lb.						
{ DDT (Seed)—one appl.	2 oz.	1.5	—	—	—	44.7	—
{ Chlordane (Soil surface)	2.5 lb.						
<i>Soil</i>							
Aldrin Emulsion	3 lb.	0.5	4.6	2.3	2.1	40.6	8
Aldrin Emulsion	6 lb.	—	—	—	1.6	52.8	22
Heptachlor Emulsion	3 lb.	—	—	2.9	—	43.9	—
<i>Drench</i>							
Aldrin Emulsion	3 lb.	—	1.8	3.6	2.3	36.7	12
Check (untreated)		3.2	6.1	6.4	5.2		62
Difference necessary for significance at 1% level		0.9	1.8	1.3	1.2		
at 5% level		0.7	1.3	0.9	0.9		

<sup>1</sup> Snedecor 1946: Table 16.8, pp. 449-450.

<sup>2</sup> Average number of plants per plot 2,500.

<sup>3</sup> For technical names, see text.



than a third. Seed treatment with dieldrin or DDT combined with one soil surface treatment with either chlordane or aldrin were very effective but were not significantly better than the seed treatments alone, and were less economical and practical. Chlordane was effective as a soil surface treatment at 4.5 pounds per acre, and is recommended where seed has not been treated. Aldrin as a soil or as a drench treatment, and heptachlor as a soil treatment only, did not give practical control.

All treatments except toxaphene gave increased yields over the checks (Table I). In 1958, based on percentage of injured bulbs taken at harvest in September, late feeding by larvae was particularly evident and may have resulted from cool weather conditions during June which retarded larval development and extended the feeding period by several days causing injury to well formed bulbs. Larval survival might also have been due to the development of a resistant strain to hydrocarbon insecticides. More than half of the bulbs in the checks showed late feeding injuries.

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# CHEMICAL CONTROL OF THE RASPBERRY ROOT BORER, *BEMBECIA MARGINATA* (HARR.), ON LOGANBERRY IN BRITISH COLUMBIA

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## ABSTRACT

A single spray of 25 per cent Diazinon emulsible concentrate applied as a drench to loganberry crowns in March, April or October against early instar larvae of the raspberry root borer, *Bembecia marginata* (Harr.) reduced a severe infestation (77 per cent or more) to 4 per cent or less of the crowns. This outstanding control was obtained at rates as low as 2 pints per 100 gallons and 1/2 pint of drench per plant (43 gallons per acre). Applications in May at 2 pints of drench per crown reduced the infestation to 10 per cent. The following emulsible concentrates reduced the infestation to between 0 and 10 per cent: at 1 pint of drench per crown applied in October, lindane at 5 pints, Thimet at 1 pint, or 12008 at 1 pint per 100 gallons; at 2 pints of drench per crown applied in April, Sevin at 8 pints, NC262 at 1/2 pint, or Phosdrin at 1 pint per 100 gallons. The drenches apparently killed the early instar larvae which overwinter in hibernacula at the base of the canes until early April, and feed just beneath the bark in May. Thus damage to canes that would bear fruit the following year was prevented.

## INTRODUCTION

*Bembecia marginata* (Harr.) is one of the most destructive pests of cane fruits in British Columbia. A 2-year study of the life history including dissection of loganberry roots dug at 2-week intervals from March to November indicates that the life history is briefly as follows: The adults appear in August and deposit their eggs singly on the undersides of the new leaves near the margin. The eggs hatch in 4 to 8 weeks and the young larvae crawl down to the base of the canes and overwinter in hibernacula formed on the canes just below soil level. The following spring they burrow in the cambium of the bearing canes undermining the new buds and commencing to girdle the new shoots arising from the base, thus reducing the number of new canes produced. During the summer they continue to feed at the base of the new canes girdling them and causing them to form gall-like swellings at the base. These canes usually become spindly and lack vigour and, although they often bear fruit, they usually break off when tied up on wires the following spring. The larvae overwinter for a second season, usually moving upwards a few inches into the young canes or the stubs of the bearing canes of the past season. During the second summer they tunnel in the fleshy part of the roots near the base of the bearing canes and in the crowns, further reducing the vigour of the plants. In July of the second season they tunnel upwards a few inches into the bearing canes or return to the overwintering tunnels of the past season and pupate, leaving a thin layer of bark at the side of the emergence tunnel which is broken open by the pupa just before the adults emerge in August. The larvae will also pupate in any part of the crown exposed above soil level, or in earthen cells made against the crown at soil level.

The present method of control in Canada consists of removing and burning injured canes during early summer and late fall (2). This method is not satisfactory as it involves a great deal of hand labour and gives only partial control.

Clark (1) and Wallace (4) obtained satisfactory control of the root borer on raspberries, using ovicides and sprays applied to the bases of canes in September. These methods are not applicable to loganberries because of the different growth habits and because numerous applications or very residual sprays are required to keep a deposit of insecticide on the canes throughout the protracted hatching period of the eggs. The approach of Gilbert *et al.* (3), using a single spray of BHC or parathion in December when the first instar larvae are overwintering in the hibernacula, appeared more promising.

This is a report of experiments conducted on southern Vancouver Island, B. C., from 1955 to 1958, to control the early instar larvae using a drench of various insecticides applied to the crowns of loganberry plants during March, April, May or October.

#### METHODS AND MATERIALS

Experiments were conducted in a planting having a history of severe root borer attack. From 1955 to 1958 a total of six randomized block experiments were established. Within any one block, the plots for each treatment consisted of a minimum of four plants and each plot was replicated at least four times. The total number of treated crowns are recorded in Table 1. The number of gallons per acre is calculated on the basis of 690 plants per acre.

The drenches were applied to the crowns and the bases of the canes on October 3, 1955, October 11, 1956, April 1, 1957 and March 27, April 3 and May 22, 1958. They were directed against the early instar larvae overwintering in hibernacula from October to early April or feeding just beneath the bark at the base of the new canes in May. For drenches applied in October, the canes and foliage were pulled aside to expose the crowns. All drenches were emulsible concentrates in water and were applied at 100 pounds pressure. The insecticides that were effective and their amounts are given in Table 1.

In 1955 and 1956, the drenches were applied with a hand gun having a disk-type nozzle\* (orifice diameter .063 inches), and in 1957 and 1958 with a 3-foot spray wand having a quick shut-off valve and a solid-cone nozzle\*\* (Tee Jet 1/4 TG. 3).

These nozzles were held about 15 inches above the crowns and applied about 1 pint of drench in 10 seconds.

Since it was not practicable to dig plants in a commercial planting and count the number of borers within the roots, the crowns were examined for piles of frass and canes that exhibited galls at the base (Figure 1). When such canes were found, the larvae were exposed by cutting into the

\*John Bean Division, Food Machinery and Chemical Corp., Lansing, Mich.

\*\*John Brooks and Co., Ltd., Montreal, Que.

TABLE 1.—LOGANBERRY CROWNS INFESTED WITH LARVAE OF *B. marginata* IN OCTOBER AFTER THE CROWNS WERE DRENCHED IN MARCH, APRIL, MAY, OR OCTOBER WITH EMULSIBLE CONCENTRATES OF VARIOUS INSECTICIDES, 1955-58

Insecticide formulation <sup>1</sup>	Imperial pint per 100 gal.	Total no. crowns	Percentage infested <sup>2</sup>
<i>1 pint of drench per crown<sup>3</sup> applied October 3, 1955</i>			
Diazinon, 25%	5	30	0
Lindane, 4%	5	36	6
Untreated	—	36	81
<i>1 pint of drench per crown applied October 11, 1956</i>			
Diazinon, 25%	3	16	0
Thimet, 90%	1	16	0
12008, 48.5%	1	16	6
Untreated	—	15	96
<i>2 pints of drench per crown applied April 1, 1957</i>			
Diazinon, 25%	3	81	4
Untreated	—	81	91
<i>1 or 1/2 pint of drench per crown applied March 27, 1958</i>			
Diazinon, 25%	2	20	0
Diazinon, 25%	2	20	0
Untreated	—	21	86
<i>2 pints of drench per crown applied April 3, 1958</i>			
Diazinon, 25%	2	44	2
Sevin, 13%	8	40	8
NC262, 40%	1/2	36	8
Phosdrin, 25%	1	40	10
Untreated	—	39	77
<i>2 pints of drench per crown applied May 22, 1958</i>			
Diazinon, 25%	2	20	10
Untreated	—	19	84

<sup>1</sup>*Diazinon*, O, O-diethyl O-(2 isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate; *Lindane*, 1, 2, 3, 4, 5, 6-hexachlorocyclohexane, 99% or more gamma isomer; *Thimet*, O, O-diethyl S-(ethylthio) methyl phosphorodithioate; *12008*, O, O-diethyl S-(isopropylthio) methyl phosphorodithioate; *Sevin*, 1-naphthyl N-methylcarbamate; *NC 262*, O, O-dimethyl S (N-methyl carboxamide methyl) phosphorothiolothionate; *Phosdrin*, Alpha isomer 2-carbomethoxy-1-methylvinyl dimethyl phosphate.

<sup>2</sup>By X<sup>2</sup>, all treatments are significantly different from their respective checks @ .01 level.

<sup>3</sup>1 pint of drench per crown = 86 gallons per acre based on 690 plants per acre.

galls. The canes were examined in October of the same year for drenches applied in March, April, or May, and in October of the following year for drenches applied in October. The frass piles and galls were caused by 1-year-old larvae feeding at the base of the young canes and adjoining crown, the second-year larvae having pupated and emerged as moths in August. The absence of frass piles and galls in the treated plots indicated mortality of first-stage larvae the previous spring or fall. The total number of infested plants per treatment were recorded and the data analysed by the X<sup>2</sup> method (Table 1).

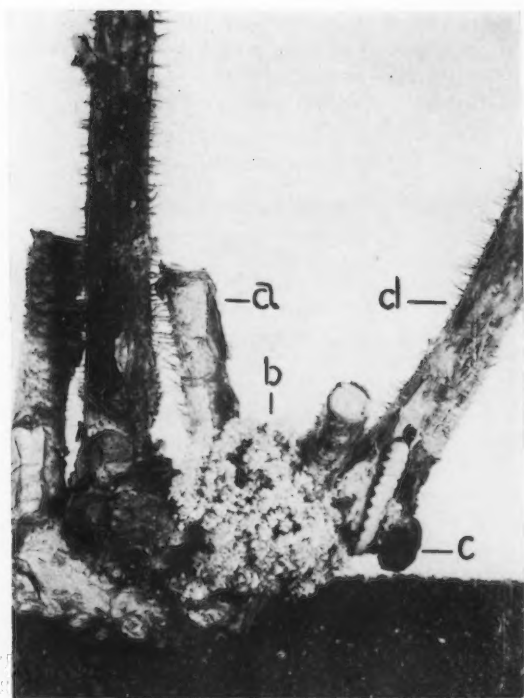


FIGURE 1. Loganberry root infested by the raspberry root borer in October. Stub of old, or past seasons' fruiting cane at (a); frass pile (b) and gall (c), caused by 1-year-old larva feeding at the base of new, or next season's fruiting cane (d).



To determine if the vigour of plants was increased when larvae were controlled, the canes of 80 plants drenched with Diazinon in April, 1957, were weighed in November, 1958, and the weights compared with those of a similar number of plants not drenched. The weight of canes after harvest was selected rather than fruit yields because cane weights were more convenient to obtain on grower property and because the Experimental Farm, Research Branch, Canada Department of Agriculture, Saanichton, B. C.,\* has found this method to be as accurate as fruit yields in evaluating fertilizer trials.

To determine if the number of canes that would bear fruit was increased when larvae were controlled, 48 plants were drenched with Diazinon in April, 1958, and the number of canes produced and infested compared in November, 1958, with those of a similar number of plants not drenched.

### RESULTS AND DISCUSSION

Table 1 includes only those insecticides which reduced infestations to less than 10 per cent of the crowns. All the insecticides listed in the table gave a highly significant reduction in the percentage of infested crowns compared with untreated crowns. There was no significant difference between any of the insecticides in the table. There was no significant difference between the Diazinon treatments applied in March, April, May, or October. However, the drenches of late March and early April were easier to apply, the canes being without foliage and tied up on wires.

Diazinon at 2 pints per 100 gallons, applied at 1, 1/2, 1/5 or 1/10 pint of drench per crown on March 27, 1958, allowed no infested crowns at the 1 or 1/2 pint volumes (Table 1) and reduced the infestation to 19 and 17 per cent of the crowns at the 1/5 and 1/10 volumes, respectively, compared to 86 per cent for the untreated crowns.

The canes of the 80 plants drenched with Diazinon in April, 1957, and weighed in November, 1958, averaged 18 ounces per plant; those not drenched, 12 ounces. The difference in weight was significant and the increase in plant vigour was attributed to control of the early-instar larvae which normally would have over-wintered in 1957-58 and damaged the 1958 canes. The 48 plants drenched in April, 1958, produced an average of 11 canes per plant, none of which was infested; those not drenched produced an average of 9 canes per plant, 1 of which was infested and broke off. Thus a single drench of Diazinon in April prevented root borer damage and increased the number and vigour of the canes. The effect of drenches at other times of the year on number and vigour of canes was not measured.

Other materials\*\* that significantly reduced the number of infested crowns, but allowed more than 20 per cent, were: emulsible concentrates of Systox, TEPP or nicotine at 1 pint per 100 gallons and 1 pint of drench

\*Personal communication.

\*\*Systox 50%, mixture of O, O-diethyl S-(2-ethylthio) ethyl phosphorothioate and O, O-diethyl O-(2-ethylthio) ethyl phosphorothioate; TEPP 20%, tetraethyl pyrophosphate; nicotine 40% alkaloid, 1,1-methyl-2 (3 pyridyl)-pyrrolidine, "Black Leaf 40", plus 1/2 lb. washing soda; DDT 25%, dichloro diphenyl trichloroethane; Guthion 18.4% O, O-dimethyl S-(4-oxo-3 H-1, 2, 3-benzotriazine-3-methyl) phosphorodithioate; malathion 50%, O-O-dimethyl phosphorodithioate, S-ester with diethyl mercaptosuccinate; Trithion 47.3%, O, O-diethyl S-(p-chlorophenylthio) methyl phosphorodithioate; heptachlor 25%, 1(or 3a), 4, 5, 6, 7, 8, 8-heptachloro-3a, 4, 7, 7a-tetrahydro-4, 7-methanoindene.



per crown; DDT at 3 pints, Guthion at 1 pint, malathion at 4 pints or Trithion at 3 pints per 100 gallons and 2 pints of drench per crown. Of these, Systox and TEPP appeared promising when applied in October. Heptachlor at 2 pints per 100 gallons and 2 pints per crown was not effective in either April or October.

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# SOIL INSECTICIDES FOR CONTROL OF THE TUBER FLEA BEETLE, *EPITRIX TUBERIS* (GENT.), IN THE INTERIOR OF BRITISH COLUMBIA<sup>1</sup>

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## ABSTRACT

In the interior of British Columbia, single treatments of aldrin, chlordane, dieldrin or heptachlor applied before or soon after planting at 4.0, 7.5, 1.5 and 3.0 pounds of toxicant per acre, respectively, and immediately incorporated into the soil by disking or harrowing were highly effective against the tuber flea beetle. These soil treatments were more reliable, economical and convenient for controlling this pest than were the numerous DDT foliage treatments.

## INTRODUCTION

The first record of the tuber flea beetle, *Epitrix tuberis* Gent., in British Columbia was made in the lower Fraser Valley in 1940 (6). By 1944, it was present in the southern interior of the province (12). In both areas the spread was rapid. Damage caused by this pest resulted in heavy financial losses not because of reduced yields but because larval feeding resulted in tubers that could not be marketed.

In an attempt to eliminate or reduce tuber flea beetle damage, insecticide control experiments were conducted in the Fraser Valley and in the interior of British Columbia. Early experiments, designed to kill adults on the foliage, showed that applications of DDT<sup>2</sup> and calcium arsenate reduced damage (6). By 1948, it had been shown that carefully applied and properly timed applications of DDT alone provided a reasonably satisfactory control (4, 5). However, the labour and machinery involved in the numerous applications proved costly. In coastal regions, where late blight, *Phytophthora infestans* (Mont.) DeBy., is a serious disease of potatoes, the cost of foliage treatments can be reduced by combining DDT with the necessary fungicide. A combined treatment is not used in the interior of B.C. because late blight is uncommon. Moreover, the necessity of frequent irrigation in this region makes the operation of ground equipment for applying foliage treatments difficult.

Preliminary investigations by Neilson at Kamloops in 1950<sup>3</sup>, indicated that certain insecticides incorporated into the soil controlled the tuber flea beetle and overcame some of the disadvantages of the DDT foliage treatment. From 1951 to 1955 the writer conducted further investigations to determine the most effective insecticides, the minimum effective rates and the best equipment for incorporating the insecticides.

<sup>1</sup> Contribution No. 5, Entomology Laboratory, Research Branch, Canada Department of Agriculture, Kamloops, B.C.

<sup>2</sup> 1-1-1-trichloro-2, 2-bis (p-chlorophenyl) ethane.

<sup>3</sup> Neilson, C. L. Tuber flea beetle control in the interior of British Columbia. Monthly Report, Entomology Laboratory, Kamloops, B.C. Unpublished. Sept., 1950.

TABLE 1.—METHODS OF TREATMENT AND DESIGN OF EXPERIMENTS FOR CONTROL OF THE TUBER FLEA BEETLE IN THE INTERIOR OF BRITISH COLUMBIA IN 1951, 1952, 1953 AND 1955

Experiment number and year	A. 1951	B. 1952	C. 1953	D. 1953	E. 1955	F. 1955
Location	Kamloops	Cache Creek	Cache Creek	Kamloops	Kamloops	Lavington
Plot size	1/17-acre	1/24-acre	1/40-acre	1/40-acre	1/60-acre	1/60-acre
Plot design	Randomized block	Randomized block	Randomized block	Randomized block	Randomized block	Randomized block
No. replicates	3	4	4	4	4	4
Application equipment	Hand duster and orchard sprayer	Hand duster and knapsack sprayer	Hand duster and orchard sprayer	Hand duster and orchard sprayer	Orchard sprayer	Orchard sprayer
Time of application	Pre-planting	Post-planting	Post-planting	Pre-planting	Post-planting	Pre-planting
Method of incorporation	Harrowing	Harrowing	Harrowing	Disking	Disking	Disking
Sample size	36 hills	36 hills	24 hills	24 hills	24 hills	24 hills
Subsample size	36 hills	25 lb.	20 lb.	20 lb.	20 lb.	20 lb.

## MATERIALS AND METHODS

During the period 1951 to 1955, six tuber flea beetle control trials were conducted at Kamloops, Cache Creek and Lavington in the dry belt region of British Columbia. The latter two locations are 51 miles west and 82 miles southeast of Kamloops, respectively. The heavy clay loam soil types and general climatic conditions, excepting annual precipitation, are similar for all points. The average annual precipitation at Lavington is 20 to 30 inches, whereas at Kamloops and Cache Creek it is 5 to 10 inches. The various locations, plot sizes, plot design, and other pertinent information, are shown in Table I.

The insecticides, formulations, types of treatment and rates of toxicant per acre which were tested in the various experiments are shown in Table 2.

Following preparation of the seed bed, insecticides were applied to the plots and incorporated into the soil at convenient times before or after seed-piece planting. Post-planting treatments were applied in time to prevent tillage machinery used to incorporate the insecticide from damaging emerging plants.

TABLE 2.—INSECTICIDES TESTED AGAINST THE TUBER FLEA BEETLE IN THE INTERIOR OF BRITISH COLUMBIA IN 1951, 1952, 1953 AND 1955

Experiment No.	Year	Insecticide	Formulation	Treatment	Toxicant per acre
A	1951	DDT <sup>1</sup>	5% Dust	Foliage	2 Applic. 1.0 lb. and 4 Applic. 1.5 lb.
		Dieldrin <sup>2</sup>	1% Dust	Soil	2.5 lb.
		Dieldrin	18½% Emulsifiable conc.	Soil	2.5 lb.
B	1952	Aldrin <sup>3</sup>	2½% Dust	Soil	3.0 lb.
		DDT	5% Dust	Foliage	2 Applic. 1.0 lb. and 4 Applic. 1.5 lb.
		Dieldrin	1½% Dust	Soil	2.5 lb.
C, D	1953	Dieldrin	18½% Emulsifiable conc.	Soil	2.5 lb.
		Aldrin	20% Emulsifiable conc.	Soil	2.0 lb.
		Aldrin	20% Emulsifiable conc.	Soil	4.0 lb.
		Chlordane <sup>4</sup>	5% Dust	Soil	10.0 lb.
		DDT	5% Dust	Foliage	2 Applic. 1.0 lb. and 4 Applic. 1.5 lb.
E, F	1955	Dieldrin	15% Emulsifiable conc.	Soil	2.0 lb.
		Aldrin	20% Emulsifiable conc.	Soil	3.0 lb.
		Aldrin	20% Emulsifiable conc.	Soil	4.0 lb.
		Chlordane	65.5% Emulsifiable conc.	Soil	7.5 lb.
		Chlordane	65.5% Emulsifiable conc.	Soil	10.0 lb.
		Dieldrin	15% Emulsifiable conc.	Soil	1.0 lb.
		Dieldrin	15% Emulsifiable conc.	Soil	1.5 lb.
		Heptachlor <sup>5</sup>	20% Emulsifiable conc.	Soil	3.0 lb.
		Heptachlor	20% Emulsifiable conc.	Soil	4.0 lb.

<sup>1</sup> 1, 1, 1-trichloro-2, 2-bis (p-chlorophenyl) ethane

<sup>2</sup> 1, 2, 3, 4, 10, 10-hexachloro-6, 7-epoxy-1, 4, 4a, 5, 6, 7, 8, 8a-octahydro-1, 4-endo-exo-5, 8-dimethanonaphthalene

<sup>3</sup> 1, 2, 3, 4, 10, 10-hexachloro-1, 4, 4a, 5, 8, 8a-hexahydro-1, 4-endo-exo-5, 8-dimethanonaphthalene

<sup>4</sup> 1, 2, 4, 5, 6, 7, 8, 8-octachloro-2, 3, 3a, 4, 7, 7a-hexahydro-4, 7-methanoindene

<sup>5</sup> 3a, 4, 5, 6, 7, 8, 8-heptachloro-3a, 4, 7, 7a-tetrahydro-4, 7-methanoindene

Foundation "A" Netted Gem seed potatoes were planted in all experimental plots. This variety is the most commonly grown main-crop potato in the interior of British Columbia.

Insecticides were applied to the soil surface with either hand dusters, knapsack or power-driven orchard sprayers and incorporated by harrowing or disking in two directions at right angles to one another. With two exceptions, insecticides were incorporated immediately after application. In 1951, 1 day elapsed and, in 1952,  $2\frac{1}{2}$  days elapsed before the insecticides were incorporated into the soil.

DDT foliage treatments were applied according to the recommendations of Finlayson and Neilson (4). DDT 5 per cent dust was applied with hand dusters starting when the plants were 2 inches high. The first two

TABLE 3.—EFFECTIVENESS OF VARIOUS SOIL-INCORPORATED INSECTICIDES FOR CONTROL OF THE TUBER FLEA BEETLE IN THE INTERIOR OF BRITISH COLUMBIA IN 1951, 1952, 1953, AND 1955, AS INDICATED BY THE AVERAGE PERCENTAGES OF MARKETABLE TUBERS<sup>1</sup>

Treatment	Actual toxicant per acre	Percentage market- able tubers <sup>2</sup> (according to site and year)	
		A. 1951	B. 1952
Dieldrin 18½% emul. incorp. into soil	2.5 lb.	88.8	77.3
Dieldrin (1% dust 1951) (1½% 1952) incorp. into soil	2.5 lb.	87.7	71.2
DDT 5% dust foliage application	{2 applic. 1.0 lb. 4 applic. 1.5 lb.	52.9	63.1
Aldrin 2½% dust incorp. into soil	3.0 lb.	—	52.0
CHECK	—	4.9	0.0
L.S.D. at 5% level	—	19.1	22.1
		C. 1953	D. 1953
Dieldrin 15% emul. incorp. into soil	2.0 lb.	99.0	89.4
Aldrin 20% emul. incorp. into soil	4.0 lb.	99.4	80.1
Chlordane 5% dust incorp. into soil	10.0 lb.	93.7	88.5
Aldrin 20% emul. incorp. into soil	2.0 lb.	89.0	49.8
DDT 5% dust foliage application	{2 applic. 1.0 lb. 4 applic. 1.5 lb.	97.3	11.4
CHECK	—	57.4	17.0
L.S.D. at 5% level	—	22.0	20.8
		E. 1955	F. 1955
Heptachlor 20% emul. incorp. into soil	4.0 lb.	98.7	100.0
Heptachlor 20% emul. incorp. into soil	3.0 lb.	93.6	100.0
Aldrin 20% emul. incorp. into soil	4.0 lb.	93.2	100.0
Chlordane 65.5% emul. incorp. into soil	7.5 lb.	88.1	100.0
Dieldrin 15% emul. incorp. into soil	1.5 lb.	83.4	97.2
Chlordane 65.5% emul. incorp. into soil	10.0 lb.	81.1	100.0
Aldrin 20% emul. incorp. into soil	3.0 lb.	75.0	100.0
Dieldrin 15% emul. incorp. into soil	1.0 lb.	62.2	99.6
CHECK	—	1.0	45.0
L.S.D. at 5% level	—	14.9	12.8

<sup>1</sup> Tubers of marketable size having 9 or fewer *E. tuberis* larval tunnels

<sup>2</sup> Lines indicate no significant differences between treatment averages according to Duncan's New Multiple Range Test (3)

applications were made at the rate of 1.0 pound of toxicant per acre at a 7-day interval. The following four applications were made at the rate of 1.5 pound of toxicant per acre at 10-day intervals.

At harvest, a sample of tubers was obtained from each plot. In 1951, a 36-hill sample was dug from each plot and used in the final assessment. In 1952, 25-pound subsamples of marketable-sized tubers were selected at random from the original 36-hill samples. In 1953 and 1955, 20-pound subsamples of marketable-sized tubers were selected at random from 24-hill plot samples. In each plot, samples were dug from the rows on a diagonal pattern leaving a 3-foot buffer zone within the border.

To determine treatment effectiveness, each tuber in a subsample was hand peeled and the number of larval tunnels recorded in the following groups: 0; 1-4; 5-9; 10-14; 15-19; 20 and over. Tubers having 9 or fewer larval tunnels were considered to be marketable while those having 10 or more were not.

### RESULTS AND DISCUSSION

Results from foliage and soil-incorporated insecticide treatments applied against the tuber flea beetle are summarized in Table 3.

Tuber flea beetle infestations varied considerably from one experimental site to another. This is indicated by the variations in average percentages of marketable tubers in the checks. For example, in (C) 1953, 57.4 per cent of the tubers in the checks were marketable, whereas in (B) 1952, none was marketable.

In (A) 1951 and (D) 1953, the foliage treatment of DDT 5 per cent dust was significantly less effective than the soil-incorporated insecticides but in (B) 1952, and (C) 1953, the DDT treatment did not differ significantly from the soil-incorporated insecticides. The ineffectiveness of the DDT foliage treatment indicated in experiments (A) and (D) may have been due in part to one or more of the following factors:

(i) Failure to obtain thorough coverage. Poor coverage during one or more of the six DDT dustings would permit the beetles to feed on untreated potato foliage. Kring (9) showed that the potato flea beetle, *Epirix cucumeris* Harris, preferred untreated to treated foliage.

(ii) New plant growth. New growth also provides an uncontaminated food source for the beetles between DDT treatments.

(iii) Failure to closely synchronize the DDT applications with beetle emergence. If emergence of any one generation were concentrated over a period of a few days rather than spread out over a period of several weeks as normally occurs, mating could take place and oviposition begin before the next (DDT) application. Neilson and Finlayson (12) showed that mating of the tuber flea beetle occurs almost immediately following emergence and the preoviposition period for the two generations averages 6 days.

(iv) Weathering of DDT. Each of the DDT treatments was exposed to weathering for periods of 7 or 10 days. It is probable that effectiveness was substantially reduced by the high summer temperatures which prevailed, and which are normal in the southern interior of British Columbia. As indicated by various workers, including Cullinan (2) and Gunther and Tow (7), dehydrohalogenation of DDT occurs under field conditions and is hastened at higher temperatures. In addition, Guthrie (8) showed that the toxicity of DDT is reduced at higher temperatures in contrast to some of the other chlorinated hydrocarbon insecticides.

Aldrin  $2\frac{1}{2}$  per cent dust or 20 per cent emulsifiable concentrate at 2.0 or 3.0 pounds of toxicant per acre provided economic control. Aldrin at the 4.0-pound rate in (D) 1953 and (E) 1955 was significantly more effective.

Chlordane 5 per cent dust or 65.5 per cent emulsifiable concentrate was highly effective when applied at 7.5 and 10 pounds of toxicant per acre. Dieldrin 1 or  $1\frac{1}{2}$  per cent dust, or 15 or  $18\frac{1}{2}$  per cent emulsifiable concentrate was highly effective at 1.5-, 2.0- and 2.5-pound rates but only moderately effective at the 1.0-pound rate. Heptachlor 20 per cent emulsifiable concentrate was highly effective at the 3.0- and 4.0-pound rates.

Soil-incorporated treatments of dieldrin in (A) 1951, and dieldrin or aldrin in (B) 1952, were less effective than the same materials tested in later experiments. This occurred in spite of the fact that the earlier dieldrin treatments were applied at higher rates. The lower effectiveness of these treatments was probably due to the time which elapsed between insecticide application and incorporation into the soil; in (A) 1951, 1 day elapsed, and in (B) 1952,  $2\frac{1}{2}$  days elapsed before the insecticides were incorporated. In the other experiments, the insecticides were incorporated into the soil almost immediately after application, to reduce the rate of volatilization or chemical breakdown.

The residual action of the soil insecticides provides protection against first and second generation tuber flea beetles. It is assumed that eggs, larvae and adults are destroyed in the soil. In contrast, the DDT treatment is effective against the beetles on the foliage only. In the experiments, soil treatments of dusts or emulsifiable concentrates worked equally well although the latter were more convenient to apply and less subject to wind drifting.

Pre- or post-planting applications of soil insecticides also worked equally well. Post-planting treatments must be applied soon after planting so tillage machinery used to incorporate the insecticide will not damage emerging plants and thereby prevent thorough incorporation.

In these experiments the soil insecticides were applied with hand dusters and knapsack or power-driven orchard sprayers. However, numerous observations of work done by farmers indicate that weed sprayers, fertilizer spreaders or seed drills are as effective as commercial dusters or sprayers for applying soil insecticides.

Since there was no difference in insecticidal effectiveness attributable to methods of incorporation, it would appear that disking and harrowing were equally effective in thoroughly mixing the insecticide into the soil. The degree of effectiveness suggests that incorporation deeper than the 2 to 4 inches obtained with these implements\* (10) is unnecessary. This substantiates work by Banham and Handford (1) and Fulton (5) but differs from that of Morrison and Crowell (10, 11) who showed incorporation to depths of 6 inches was necessary for effective control in Oregon.

Soil treatments of aldrin, chlordane, dieldrin or heptachlor were more economical than foliage treatments of DDT. Although the cost of insecticide for each treatment is approximately \$20.00 per acre per season, only one application of soil insecticide is required instead of five or more applications of DDT.

\* Neilson, C. L. Tuber flea beetle control in the interior of British Columbia. Monthly Report, Entomology Laboratory, Kamloops, B.C. *Unpublished*. May, 1950.



Field observations in the experimental plots during the growing season and at harvest indicated that aldrin, chlordane, dieldrin or heptachlor soil treatments applied against the tuber flea beetle effectively controlled other common soil inhabiting insect pests such as wireworms *Elateridae* spp., white grubs mainly *Phyllophaga anixia*, and cutworms, mainly *Euxoa ochrogaster* and *Euxoa messoria* as well as the tuber flea beetle.

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# SPRINKLER IRRIGATION AS A MEANS OF APPLYING SOIL INSECTICIDES FOR CONTROLLING THE TUBER FLEA BEETLE, *EPITRIX TUBERIS* (GENT.), IN THE INTERIOR OF BRITISH COLUMBIA<sup>1</sup>

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## ABSTRACT

Dieldrin emulsifiable concentrate, applied through sprinkler irrigation systems at the rate of 2 or 2.5 pounds of toxicant per acre and incorporated into the soil by disking or harrowing, effectively reduced tuber flea beetle damage in the interior of British Columbia. Similar treatments of aldrin emulsifiable concentrate applied at the rate of 4 pounds of toxicant per acre were much less effective. Colorimetric and bioassay analyses indicated that mechanical incorporation was necessary because no appreciable amount of toxicant penetrated below the top inch of soil even when a large amount of irrigation water was applied.

## INTRODUCTION

With the increased use of sprinkler irrigation systems throughout the southern interior of British Columbia, this equipment suggested itself as a more economical and efficient means of applying insecticides for tuber flea beetle control than the conventional sprayers or dusters. Since applications of soil insecticides against this pest could be effectively made either before or after planting but prior to foliage emergence (2, 3), it was evident that their application could be synchronized with an early spring irrigation.

Following exploratory investigations in 1951, more formal experiments were conducted in 1952, 1953 and 1954. The methods and experimental designs which differed considerably from year to year are described below.

## MATERIALS AND METHODS

### *1951 Experiment*

Exploratory work in 1951 was conducted at Kamloops, British Columbia. One application of aldrin<sup>2</sup> was made during a single setting of a sprinkler head to an area over which 40 cage bases were distributed. These consisted of open-ended, galvanized iron cylinders, 10 inches in diameter by 12 inches deep and were set out along eight equidistant radii at points 5, 10, 15, 20 and 25 feet from the sprinkler head. Each was buried to a depth of 8 inches. Cage bases along four of the eight radii were covered during treatment. Thus, alternate radii had check or treated cages.

Prior to the application of aldrin, Foundation "A" Netted Gem potatoes were planted in each of the 40 cage bases. After treatment a lumite top measuring 12 by 12 by 36 inches high was attached to each metal cage base.

Aldrin 23 per cent emulsifiable concentrate was applied at 3 pounds of toxicant per acre through a Rainbird Model 20A sprinkler head equipped with a 20°, 5/32-inch nozzle and operated at a pressure of 25 pounds per

<sup>1</sup> Contribution No. 6, Entomology Laboratory, Research Branch, Canada Department of Agriculture, Kamloops, B.C.

<sup>2</sup> 1, 2, 3, 4, 10, 10-hexachloro-1, 4, 4a, 5, 8, 8a-hexahydro-1, 4-endo-exo-5, 8-dimethanonaphthalene.

square inch. At this pressure the sprinkler covered an area with a radius of 28 feet (1/19-acre), and discharged 3.43 gallons per minute (1). The aldrin was applied over a period of 1 hour immediately following an irrigation period of 6 hours.

Aldrin was introduced into the sprinkler system by means of a specially designed, pressurized injector<sup>1</sup>. This was equipped with a cylinder of compressed oxygen attached to a differential pressure gauge, nozzle, and a 4-gallon insecticide tank. This arrangement permitted the insecticide to be injected into the irrigation system at a constant pressure and uniform rate over a given period of time. The injector was attached to the intake side of an electrically driven rotary irrigation pump so the injected insecticide would be thoroughly mixed into the irrigation water by the rotation of the pump impeller.

Ten pairs of first-generation tuber flea beetles, taken in the field in copulation, were put into each cage; five pairs on June 28 and five on July 4. At harvest, the potatoes in each cage were dug, hand peeled, and the total number of larval tunnels noted (2).

At harvest, soil samples were taken to determine the distribution pattern of the aldrin from the point at which the sprinkler head was operated and the depth of penetration. To determine the distribution pattern, soil cores 6 inches in diameter by 3 inches deep were taken along each of four equidistant radii at points 5, 10, 15, 20 and 25 feet from the point at which the sprinkler was operated. To determine depth of aldrin penetration, soil cores 6 inches in diameter were taken at depths of 0-1, 1-3 and 3-6 inches on each of eight equidistant radii 10 feet from the point at which the sprinkler was operated. The eight samples taken at each of the three depths were divided into two groups of four. Each group of four was composited in a cement mixer and a quart subsample taken. Similarly, each of the distribution pattern samples was composited and a quart subsample taken. Each subsample was sealed in a glass jar and sent for analysis to the Julius Hyman Company, Denver, Colorado.

### 1952 Experiment

The 1952 experiment was conducted at Heffley Creek, British Columbia. Natural infestations of tuber flea beetles were used rather than caged infestations as in 1951. Dieldrin<sup>2</sup> 18½ per cent emulsifiable concentrate was applied through a sprinkler system at the rate of 2.5 pounds of toxicant per acre over a period of 30 minutes, using the same pressurized injector and the same kind of sprinkler heads as were used in 1951. Two laterals of 2-inch aluminum irrigation pipe 40 feet apart were run from a main line connected to a gasoline-driven rotary irrigation pump. Dieldrin was applied to four 1/19-acre plots by sprinklers placed 80 feet apart in each line. A total of eight untreated spaces were left between and beyond the sprinkled plots to serve as checks. Application was made after planting but prior to foliage emergence. The sprinklers were run for 30 minutes before and after application to thoroughly flush the lines.

<sup>1</sup> C. Norris, Norco Machine Works, North Kamloops, B.C., designed and manufactured the injector under the direction of R. H. Handford, Officer-in-Charge of this Laboratory.

<sup>2</sup> 1, 2, 3, 4, 10, 10-hexachloro-6, 7-epoxy-1, 4, 4a, 5, 6, 7, 8, 8a, octahydro-1, 4-endo-exo-5, 8-dimethanonaphthalene.

The dieldrin was thoroughly incorporated into the soil by harrowing approximately 24 hours after application. Foundation "A" Netted Gem potatoes were planted in the experimental area.

At harvest, 27-hill tuber samples were taken from treated areas 24 by 24 feet centred on the site of each sprinkler head. Corresponding check samples were taken from similar-sized untreated areas centred between or beyond the treated areas. Based on data supplied by the sprinkler manufacturer (1) these areas received a fairly uniform application of dieldrin and water and were not affected by overlapping from adjacent sprinklers. From each plot sample a 25-pound subsample of marketable-sized tubers was selected at random, hand peeled, and the number of larval tunnels in each tuber recorded in the following groups: 0; 1-4; 5-9; 10-14; 15-19; 20 and over. Tubers having 9 or fewer larval tunnels were considered to be marketable while those having 10 or more were not (2).

#### *1953 Experiment*

The 1953 experiment was conducted at Kamloops, British Columbia. A simple gravity-flow injector was used to introduce the insecticide into the sprinkler irrigation system instead of the pressurized injector which was used in 1951 and 1952. The gravity-flow injector consisted of a 1-gallon aspirator bottle with a glass stopcock fitted into the bottom outlet. Rubber tubing was run from the stopcock to a  $\frac{1}{4}$ -inch galvanized pipe with shut-off valve attached. The  $\frac{1}{4}$ -inch pipe was welded into a large pipe coupling on the intake side of the irrigation pump. Aldrin was siphoned into the system by means of gravity and the partial vacuum created at the intake side of the irrigation pump. As with the pressurized injector, the insecticide was mixed with the irrigation water by the rotary action of the pump impeller.

Aldrin 20 per cent emulsifiable concentrate was applied through the sprinkler system to 1/19-acre plots at the rate of 4.0 pounds of toxicant per acre. One line of 2-inch pipe, with four sprinklers placed 80 feet apart, was run from the electrically driven rotary irrigation pump. Untreated spaces between and beyond the sprinkled plots served as checks. Thus, there were four treated plots and four checks. Aldrin was applied to the plots prior to planting and thoroughly incorporated by disking. At harvest, a 24-hill sample was taken from each 24- by 24-foot sampling area. From each plot sample a 20-pound subsample of marketable-sized tubers was selected at random, hand peeled, and the number of larval tunnels in each tuber noted as in 1952. In all other respects this experiment was similar to the one conducted in 1952.

#### *1954 Experiment*

The 1954 experiment was conducted at Kamloops, British Columbia, also, and was similar to the one conducted in 1953. Aldrin 20 per cent and dieldrin 15 per cent emulsifiable concentrates were applied at the rates of 4.0 pounds and 2.0 pounds of toxicant per acre, respectively. Two laterals of 2-inch pipe 40 feet apart were run from a main line connected to the irrigation pump. Three sprinklers were placed 80 feet apart in each of the laterals. The laterals were operated in succession to apply aldrin or dieldrin.

Untreated spaces were left between and beyond the sprinkled plots to serve as checks. There were three aldrin-treated plots and three checks in one line, and three dieldrin-treated plots and three checks in the other. The insecticides were applied to the plots prior to planting and thoroughly incorporated by harrowing.

## RESULTS AND DISCUSSION

### 1951 Experiment

Aldrin applied to the soil through a sprinkler system reduced tuber flea beetle damage in cages located at intervals up to 15 feet from the sprinkler site. Effectiveness decreased in proportion to the distance from the sprinkler head as shown by the following average numbers of larval tunnels per cage:

137 in treated and 911 in check cages	5 ft. from the sprinkler
454 in treated and 634 in check cages	10 ft. from the sprinkler
341 in treated and 681 in check cages	15 ft. from the sprinkler
871 in treated and 702 in check cages	20 ft. from the sprinkler
692 in treated and 866 in check cages	25 ft. from the sprinkler.

Analysis of the soil samples indicated that an average of less than  $0.10 \pm 0$  p.p.m. of aldrin were present in samples taken at points 15 feet or more from the sprinkler, an average of  $0.11 \pm 0.02$  p.p.m. in samples taken at 10 feet and  $0.42 \pm 0.49$  p.p.m. in samples taken at 5 feet. Thus, as would be expected, effectiveness of the aldrin was roughly proportional to the amount of toxicant deposited. There was close agreement between the two analytical methods used. The colorimetric phenylazide method and bioassay with houseflies were sensitive to  $0.10$  p.p.m.

No, or undetectable amounts of aldrin penetrated below the first inch of soil. At points 10 feet from the sprinkler the phenylazide and bioassay methods indicated  $0.18$  and  $0.17$  to  $0.23$  p.p.m., respectively, were present in the first inch. The filtering action of the first inch of soil apparently prevented the dispersal of the insecticide emulsion to greater depths despite the free water made available for this purpose by the heavy irrigation immediately prior to treatment.

### 1952, 1953 and 1954 Experiments

The results of these experiments are summarized in Table 1.

Soil-incorporated treatments of aldrin or dieldrin applied through sprinkler irrigation systems controlled the tuber flea beetle with varying degrees of effectiveness. Dieldrin 15 or  $18\frac{1}{2}$  per cent emulsifiable concentrate applied at the rate of 2.5 or 2 pounds of toxicant per acre in 1952 and 1954, respectively, was highly effective. Aldrin 20 per cent emulsifiable concentrate applied at the rate of 4 pounds of toxicant per acre was effective in some measure in 1953 but not significantly in 1954.

Poor incorporation of the toxicant into the soil may account for the ineffectiveness of the aldrin treatments. As previously indicated, all of the soil treatments were incorporated by disking or harrowing about 24 hours after application. However, at all of the experimental sites the saturated condition of the clay loam soils caused a "balling" during cultivation and made thorough incorporation difficult. This occurred in spite

TABLE 1.—EFFECTIVENESS OF ALDRIN AND DIELDRIN APPLIED BY MEANS OF SPRINKLER IRRIGATION SYSTEMS AND INCORPORATED INTO THE SOIL BY CULTIVATION FOR CONTROL OF THE TUBER FLEA BEETLE

Experiment	No. replicates	Treatment	Pounds toxicant per acre	Percentage marketable tubers <sup>1</sup>	L.S.D. values <sup>2</sup>
A. 1952	8	Dieldrin 18½ E.C.	2.5	81.5}	8.1
	8	Check	—	3.0}	
B. 1953	4	Aldrin 20% E.C.	4.0	26.3}	14.7
	4	Check	—	9.3}	
C. 1954	3	Aldrin 20% E.C.	4.0	91.7}	32.5
	3	Check	—	59.8}	
	3	Dieldrin 15% E.C.	2.0	88.6}	50.2
	3	Check	—	18.2}	

<sup>1</sup> Tubers having 9 or fewer tuber flea beetle larval tunnels

<sup>2</sup> Differences at 5 per cent level

of the fact that the sprinklers were operated for a total period of only 90 minutes. Unless higher rates of insecticide were used, it is improbable that incorporation could have been delayed much beyond the 24-hour period between application and incorporation which would have been necessary to permit further drying of the soil. The rapid rate at which aldrin dissipates when exposed to high summer temperatures (4) would have reduced effectiveness even more if this period had been extended.

The inexpensive gravity-flow injector used in 1953 and 1954 appeared to operate as efficiently as the more expensive pressurized injector which was used in 1951 and 1952. However, it required special attention to ensure an even rate of insecticide flow. The partial vacuum created by the irrigation pump tended to siphon the insecticide into the pump too quickly unless carefully regulated. Insecticide injected into the intake side of the electrically or gasoline-driven irrigation pumps appeared to be effectively mixed into the irrigation water. Later work by L. C. Terriere in Oregon (5) showed similar results were obtained when insecticide was introduced either before or after the irrigation pump.

### CONCLUSIONS

It is doubtful if sprinkler irrigation systems offer a practical means of applying aldrin or dieldrin for tuber flea beetle control. Colorimetric and bioassay analyses indicate that mechanical incorporation is necessary because no appreciable amount of toxicant penetrated below the top inch of soil even when a large amount of irrigation water was applied. However, the incorporation of an insecticide into a soil, especially one having a high clay content, would be impossible for many hours, in some instances up to 72 hours, if application followed a normal 8- to 10-hour irrigation. Leaving the insecticides exposed on the soil surface to high summer temperatures for such a long period of time would be undesirable. Sprinkler systems would



increase rather than decrease application costs if special pipe moves were made to apply an insecticide. Unless a new insecticide is discovered which does not have to be incorporated into the soil to achieve maximum effectiveness against the tuber flea beetle or, although less probable, an inexpensive emulsifying or wetting agent becomes available that will permit chlorinated hydrocarbon insecticides to penetrate freely into the soil to depths of 2 to 4 inches, sprinkler systems are not likely to be used extensively to apply soil insecticides in the interior of British Columbia.

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# INSECTICIDE ROW TREATMENTS FOR THE CONTROL OF WIREWORMS IN POTATOES<sup>1</sup>

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## ABSTRACT

In field plots, where about 20 per cent of the potato tubers were damaged by wireworms in untreated plots, not more than 5 per cent were damaged after band treatments with aldrin or heptachlor dusts, granules, or impregnated fertilizers, applied at 1 or 3 lb. of toxicant per acre during planting. At least 15 per cent of the tubers were damaged after band treatments with chlordane dust at the same rates. Damage after treatments with aldrin or heptachlor dusts applied in the open furrow by hand immediately before planting was similar to that after band treatments at the same rates. Where about 40 per cent of the tubers were damaged in untreated plots, 9 and 15 per cent of the tubers were damaged after band treatments with granular aldrin and heptachlor respectively, each at 3 lb. per acre; 23 and 28 per cent of the tubers were damaged after band treatments with the same insecticides at 1 lb. per acre. Thus, the treatments reduced damage enough to bring the potato crop to top table stock grade, without culling, only where potential damage was light. None of the treatments reduced wireworm numbers more than 75 per cent. None of the treatments increased the yield of tubers. Furrow treatments with aldrin and heptachlor dusts reduced yield in one of two tests; presumably because of phytotoxicity.

## INTRODUCTION

Greenwood (4) found that BHC (1,2,3,4,5,6-hexachlorocyclohexane), applied with a planter fertilizer applicator during planting, appeared to be more effective than broadcast treatments in controlling wireworms, probably *Limoni* *ectypus* (Say), in potatoes in Connecticut. Begg (1) stated that, in Ontario, insecticide applications along each side of the row during or immediately after planting were not recommended for controlling the common species of wireworms in potatoes or other crops requiring a high degree of protection. He reported also (2) that a granular insecticide, applied with a fertilizer side dressing 10 and 20 days after planting, was a good emergency control for *Limoni* *agonus* (Say) attacking early potatoes. Colbert and Callenbach (3) included a band treatment of aldrin (1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4,5,8-dimethanonaphthalene), applied on each side of the row, in their recommendations for the control of wireworms, probably *Ctenicera* spp., in potatoes in North Dakota.

Wireworm damage to potatoes in Saskatchewan can be controlled with insecticides broadcast on the soil surface and cultivated into the topsoil before planting (5). In 1954, 1955 and 1956, near Saskatoon, row treatments with aldrin and heptachlor (1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene) dusts, granules, and impregnated fertilizers, and chlordane (1,2,4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene) dusts, were tested for their effects on wireworm numbers and damage and on tuber yield.

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## METHODS

Insecticide formulations tested and methods of application of each, in 1954, 1955 and 1956, are given in Tables 1, 2 and 3, respectively. All treatments in each test were replicated four times in a randomized split-plot complete block design. Each main plot was divided into sub-plots 10 feet by 30 feet, treated with various formulations or rates of the same insecticide. In 1954 the two methods of dust application were tested in separate plot layouts in the same field.

In the band treatments the potato seed pieces were planted, and the fertilizer and insecticide applied, in one operation with a planter and attached fertilizer applicator. In the open furrow treatments dusts were sprinkled in the open furrow immediately before the seed pieces were planted by hand. Granular fertilizer (11-48-0) was applied to the insecticide-free plots at the same rates as to the insecticide-treated plots.

Potato seed pieces were planted approximately 13 inches apart and 4 inches deep in rows 30 feet long and 3 feet apart. Three rows per sub-plot were planted in 1954, 1955 and 1956; two rows per sub-plot were planted in 1957, with no further treatment, in the plots treated in 1956. Certified Warba seed pieces were used in 1954, 1955 and 1957, and certified Early Ohio seed pieces in 1956.

The tests were conducted in 1954, 1955 and 1956, in clay loam, light loam, and light sandy loam soils, respectively; wireworms in untreated plots were 68, 26 and 39 per cent *Ctenicera aeripennis destructor* (Brown), respectively. The remainder were *Hypolithus bicolor* Esch., except in 1956, when 28 per cent were *Limonius* spp. and *Aeolus* spp.

TABLE 1.—YIELDS OF POTATOES, AND WIREWORM DAMAGE AND NUMBERS AFTER BAND AND FURROW APPLICATIONS OF ALDRIN, CHLORDANE, AND HEPTACHLOR DUSTS AT PLANTING TIME. SASKATCHEWAN, 1954

Treatment	Toxicant per acre, lb.	Tubers per hill, kgm.		Undamaged tubers, %		Wireworm <sup>1</sup> tunnels per tuber		Wireworms <sup>1</sup> per 1/16,000 acre
		A <sup>2</sup>	B <sup>2</sup>	A	B	A	B	
Aldrin dust, 2 1/2%	0	1.07	1.08	80	72	0.14	0.18	1.25
	1	0.99	1.10	95	94	0.02	0.03	0.84
	3	1.02	1.04	98	94	0.02	0.03	0.29
Chlordane dust, 2 1/2%	0	0.93	1.04	79	63	0.15	0.26	1.37
	1	0.92	1.09	83	84	0.12	0.10	1.07
	3	0.74	1.14	85	89	0.14	0.06	1.06
Heptachlor dust, 2 1/2%	0	0.92	1.03	78	70	0.15	0.21	1.29
	1	1.07	0.99	96	92	0.02	0.04	0.73
	3	1.04	1.08	98	96	0.01	0.02	0.68
Difference required for significance at 5% level:								
between rates of one toxicant in A or in B		—	—	11	8	0.08	0.05	0.28
between two toxicants at one rate in A or in B		—	—	12	8	0.08	0.05	0.30

<sup>1</sup>Transformed to  $\log(x+1)$  where  $x$  is the original value

<sup>2</sup>Formulation applied from planter fertilizer applicator in bands

<sup>3</sup>Formulation sprinkled in open furrow

Wireworm damage to tubers over 1 inch in diameter was estimated. Any wireworm feeding scar that penetrated the tuber skin was recorded as a tunnel, and the tuber as damaged. Plots were sampled as follows: 1954, all hills from the centre row of each sub-plot; 1955, three hills from each outside row and four from the centre row; 1956, 20 hills from the centre row in each sub-plot; 1957, 15 hills from the two rows in each sub-plot.

Estimates of wireworm numbers were obtained from 10 cylindrical samples of soil, each 1/160,000 acre in area and approximately 10 inches deep, taken from the row areas in each sub-plot following cultivation after harvest.

The tubers examined for wireworm damage were weighed to determine yields.

### RESULTS AND DISCUSSION

In the 1954 and 1955 tests (Tables 1 and 2) only about 20 per cent of the tubers in untreated plots were damaged by wireworms. Not more than 5 per cent of the tubers were damaged after band treatments with aldrin or heptachlor dusts at 1 or 3 pounds of toxicant per acre, or granules or impregnated fertilizers at 3 pounds per acre. At least 15 per cent of the tubers were damaged after band treatments with chlordane dusts at the same rates.

Damage where dusts were sprinkled in the open furrow was similar to that where the band treatments were applied at the same rates. However, in 1954, wireworm damage in the check plots where the open furrow applications were tested was higher than in those where the band applications were tested.

TABLE 2.—YIELDS OF POTATOES, AND WIREWORM DAMAGE AND NUMBERS, AFTER BAND AND FURROW APPLICATIONS<sup>1</sup> OF ALDRIN AND HEPTACHLOR AT PLANTING TIME. SASKATCHEWAN, 1955

Treatment	Toxicant per acre, lb.	Tubers per hill, kgm.	Undamaged tubers, %	Wireworm <sup>2</sup> tunnels per tuber	Wireworms <sup>2</sup> per 1/16,000 acre
Aldrin check (fertilizer)	0	1.09	82	0.11	1.12
Aldrin-impregnated fertilizer 2%	3	1.13	97	0.02	0.51
Aldrin granules 5%	3	1.06	98	0.01	0.49
Aldrin dust 5%	3	0.91	98	0.01	0.54
Heptachlor check (fertilizer)	0	1.02	84	0.10	1.23
Heptachlor-impregnated fertilizer 2%	3	1.06	96	0.02	0.64
Heptachlor granules 2 1/2%	3	1.04	99	0.00	0.39
Heptachlor dust 2 1/2%	3	0.78	98	0.01	0.51
Difference required for significance at 5% level:					
between two formulations of one toxicant (including check)		0.15	6	0.03	0.33
between two toxicants of the same formulation		0.20	5	0.03	0.33

<sup>1</sup>Granules and impregnated fertilizers applied from planter fertilizer applicator, and dusts sprinkled in the open furrow

<sup>2</sup>Transformed to  $\log(x+1)$  where  $x$  is the original value

TABLE 3.—YIELDS OF POTATOES AND WIREWORM DAMAGE AND NUMBERS, AFTER BAND APPLICATIONS OF ALDRIN AND HEPTACHLOR AT PLANTING TIME. SASKATCHEWAN, 1956, 1957

Treatment	Toxicant per acre, lb.	Tubers per hill, kgm.		Undamaged tubers, %		Wireworm <sup>1</sup> tunnels per tuber		Wireworms <sup>1</sup> per 1/16,000 acre	
		1956	1957	1956	1957	1956	1957	1956	1957
Aldrin check (fertilizer)	0	0.41	0.19	58	55	0.25	0.26	0.68	0.90
Aldrin granules 5%	1	0.38	0.16	77	91	0.14	0.08	0.44	0.58
	3	0.36	0.18	91	91	0.05	0.05	0.41	0.48
Heptachlor check (fertilizer)	0	0.40	0.19	61	62	0.25	0.22	0.83	1.04
Heptachlor granules 2 1/2%	1	0.37	0.19	72	82	0.14	0.12	0.57	0.52
	3	0.39	0.19	85	88	0.09	0.09	0.32	0.30
Difference required for significance at 5% level:									
between 2 rates of 1 toxicant		—	—	16	15	0.10	0.14	0.47	0.29
between 2 toxicants at one rate		—	—	27	24	0.16	0.18	0.48	0.39

<sup>1</sup>Transformed to  $\log(x+1)$  where  $x$  is the original value

In the 1956 test (Table 3), approximately 40 per cent of the tubers in untreated plots were damaged. Nine and fifteen per cent of the tubers were damaged after band treatments with granular aldrin and heptachlor respectively, each at 3 pounds per acre; twenty-three and twenty-eight per cent respectively of the tubers were damaged after 1-pound rates of the above insecticides. Wireworm damage to tubers in the same plots in 1957, with no further treatment, was similar to that in 1956.

The comparative effectiveness of the treatments with tunnels per tuber as a criterion of damage was similar to that with percentage undamaged tubers as the criterion.

Thus, the treatments reduced damage enough to bring the potato crop to top table stock grade, without culling, only where wireworm damage potential was light.\*

Wireworm numbers in the 1954 band treatment plots were not sampled. All furrow treatments significantly reduced wireworm numbers. Aldrin at 3 pounds was more effective than any other treatment, and all treatments but aldrin at 1 pound were more effective than chlordane at either rate. In 1955 all band treatments significantly reduced wireworm numbers. In 1956 only heptachlor granules at 3 pounds per acre did so; in 1957, in the same plots with no further treatment, wireworm numbers were similar in treated plots to those in 1956 but higher in untreated plots. None of the band treatments in any of the tests reduced wireworm numbers more than 75 per cent.

\*Based on the author's interpretation of "Regulations with Respect to Vegetables" under the Vegetable and Honey Sales Act, Saskatchewan Gazette S1(17):366-370, April 29, 1955.

Band applications treat only a small proportion of the soil. In these tests the bands were placed in the soil approximately  $3\frac{1}{2}$  inches on either side of the row of seed pieces and at the same depth. Each band was roughly elliptical in cross-section with a maximum diameter of 2 to 3 inches, and the concentration of insecticide particles decreased from the centre outwards. Greenwood (4) and Begg (2) suggested that the success of the method depends mainly on the movement of the larvae to and from the seed pieces during the early stages of plant development. However, in the 1956 test tubers were damaged in insecticide-treated plots, even though the seed pieces were attacked and the wireworm numbers were reduced. Some of the larvae, then, did not come in contact with the insecticide bands, either because they did not move to the seed pieces or because they moved over or under the band of insecticide. It is probable that larvae coming in contact with the bands were killed. The mean concentration of toxicant within the band, with a 1 pound per acre band treatment, is approximately four times that in the top 4 inches of soil with a 5 pounds-per-acre broadcast treatment. Five pounds per acre broadcast treatments of aldrin or heptachlor (5) have given at least 90 per cent reduction of wireworm damage and numbers. The data suggest that the effectiveness of the treatments was limited by the restricted distribution of the insecticides, and that this effect may vary with wireworm activity.

None of the treatments increased tuber yield. Aldrin and heptachlor furrow applications of dust, at 3 pounds of toxicant per acre, reduced the tuber yields in 1955 (Table 2), but not in 1954 (Table 1), possibly due to differences in soil conditions. Some phytotoxicity might be expected from this method of treatment because the seed pieces were in contact with the insecticide.

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# INFLUENCE OF PERIODIC SHADING ON THE LENGTH AND SOLIDNESS OF THE INTERNODES OF RESCUE WHEAT<sup>1</sup>

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## ABSTRACT

Shading at the initiation of elongation shortened the internodes of Rescue wheat whereas shading later in the elongation period caused the internodes to elongate as much as or more than if they had not been shaded.

The solidness of the bottom Internode, 1, was reduced mainly by shading from the 2-leaf to the boot stage, while that of Internodes 2 and 3 was reduced mainly by shading from the 4-leaf to the boot stage. The solidness of Internode 4 was reduced mainly by shading from the boot to the heading stage. The solidness in the lower 3 internodes was affected by reduction in light intensity even after the boot stage. Severe lodging occurred only in stems shaded from the boot to the heading stage.

Two methods were used for rating stem solidness, namely, measurement in the split stem of proportion of the internode filled with pith, and classification of solidness of cross-sections at designated points in each internode. They were strongly correlated and appeared to give equally accurate estimates of solidness, although their degrees of sensitivity varied with the amount of stem solidness present.

## INTRODUCTION

Farstad (1) found that the stem solidness of certain lines of *Triticum aestivum* L. emend Thell., including S-615, which is one of the parents of Rescue wheat, is affected by environment. When these lines were grown under cages or in the greenhouse, their solidness was reduced. Platt (6) concluded that reduction in light intensity is the major factor in reducing stem solidness in S-615. He also stated that June is the critical month in determining stem solidness in the field, although in some cases the effect may extend into July.

The importance of stem solidness in host resistance to the wheat stem sawfly, *Cephus cinctus* Nort., has been shown (1, 3, 7). The present study was conducted to determine the effect of shading during several periods in the growing stage on the solidness of each internode of Rescue, a sawfly-resistant bread wheat. In addition there was a need for a comparison of two techniques of measuring stem solidness, one based on the diameter and the other on the length of the pith cavity.

## METHODS AND MATERIALS

The experiment was conducted on dry land at Lethbridge, Alberta, in 1956. Rescue wheat was seeded on May 16 in rows spaced 7 inches apart in six blocks. Each block consisted of six randomized plots. The blocks and the plots were separated by 10 feet. Each plot was covered during a specified period with a cage approximately 2 feet square and 3 feet high. The top, east, and west sides of each cage were completely covered with heavy, unbleached cotton sheeting. To allow air movement within the cage, the north side was not covered and the south side was covered to within 6 inches of the base of the cage.

<sup>1</sup>Contribution from the Entomology Section.



The ripe wheat from each plot was collected and divided at random into two lots. Ten main stems from the first lot were split longitudinally. The length of each of the internodes, which were numbered consecutively from the base, and of its pith cavity were used to obtain percentage solidness. The data were converted by the angular transformation and subjected to the "F" test (2). Ten main stems from the second lot were rated for solidness by Larson's method (4, 5). Cross-sectional cuts were made at the centre in all five internodes as well as at 1 cm. from the top in Internodes 1 and 2 and at 2 cm. from the top in Internodes 3 and 4, and at 5 cm. from the top in Internode 5. The population shaded from boot to heading stage was not examined as the stems were too brittle. The solidness was rated by index numbers ranging from "1" for thin-walled hollow to "5" for completely solid cuts. The means for the various cuts were calculated to 0.01. Only data from the centre cuts were included in Table 3, as they were generally representative of the data from the other cuts.

## RESULTS AND DISCUSSION

### *Internode Length*

Shading the plants during different periods in their development did not exert a uniform effect on the length of the various internodes; some were elongated as expected, but others were shortened (Table 1). Hence, it appeared that stage of development of the stems during shading may have been an important factor. The mean lengths in millimeters of 10 unshaded main stems at each stage were:—

Date	Stage	Internode				
		1	2	3	4	5
June 18	4-leaf	18	4	1	0.3	—
July 6	Boot	45	90	110	23	7
Sept. 4	Mature	59	85	147	242	483

Shading before the 2-leaf stage had no effect on internode length (Table 1). Shading from the 2-leaf to the 4-leaf stage shortened Internodes 1 to 4; all of these internodes started elongating during this period (see leader table above). Shading from the 4-leaf to the boot stage lengthened Internodes 1, 2, and 3 and shortened Internodes 4 and 5; during this period elongation was completed in Internodes 1 and 2, about 75 per cent completed in Internode 3, about 10 per cent completed in Internode 4, and just started in Internode 5. Shading from emergence to the boot stage had a similar effect except that it shortened Internode 3; during this period Internode 3 was shaded during initial elongation but, apparently, not long enough during its later development to overcome the initial shortening effect. Shading from the boot to the heading stage did not affect the length of Internodes 1, 2, and 3 but it did lengthen Internode 4 and shorten Internode 5; during this period Internode 4 completed its elongation while Internode 5 had not finished when the cages were removed.



Hence, shading during the initiation of elongation of an internode reduced its elongation whereas shading later in the elongation period caused the internode to elongate as much as or more than if it had been unshaded.

### Internode Solidness

Shading up to the 2-leaf stage had no effect on internode solidness (Table 2 and 3). The most critical period in the determination of solidness in Internodes 1, 2, and 3 was from the 4-leaf to the boot stage (June 18 to July 6), although the solidness of these internodes continued to be affected by shading even after the boot stage. The most critical period in the determination of solidness in Internode 4 and probably Internode 5 was from the boot to the heading stage (July 6 to 17). Shading from the 2-leaf to the 4-leaf stage (May 29 to June 18) increased the length but not the diameter of the pith cavity of Internode 1 and increased the effect on Internodes 1 and 2 of shading continued to the boot stage. Shading between the 4-leaf and boot stages increased the solidness in the middle of Internode 4 (Table 3), and decreased the solidness in the upper part. The mean solidness indices of the upper cuts in this internode of stems shaded from emergence to the boot stage and from the 4-leaf to the boot stage were 2.95 and 2.65, which were both significantly more hollow at the 1 per cent level

TABLE 1.—THE EFFECT OF SHADING ON INTERNODE LENGTH (MM.) IN RESCUE

Dates shaded	Stage of plant shaded	Internode				
		1	2	3	4	5
None (check)	None	59	85	147	242	483
May 23 to May 29	Emergence to 2-leaf	56	84	147	246	484
May 29 to June 18	2-leaf to 4-leaf	39**	72**	119**	231*	457
June 18 to July 6	4-leaf to boot	71†	124††	161††	157**	416**
May 23 to July 6	Emergence to boot	68	120††	117**	171**	406**
July 6 to July 17	Boot to heading	61	92	148	252†	423**

\*Significantly lower than check

†Significantly higher than check

TABLE 2.—THE EFFECT OF SHADING ON PERCENTAGE SOLIDNESS BASED ON LONGITUDINALLY SPLIT STEMS OF RESCUE

Stage of plant shaded	Internode				
	1	2	3	4	5
None (check)	88	95	91	18	3
Emergence to 2-leaf	94	97	97	19	4
2-leaf to 4-leaf	66**	93	84	25	3
4-leaf to boot	30**	25**	12**	24	19††
Emergence to boot	14**	7**	16**	20	14††
Boot to heading	72*	85**	61**	3**	0

\*Significantly lower than check

†Significantly higher than check

TABLE 3.—THE EFFECT OF SHADING ON THE SOLIDNESS INDICES OF THE CENTRE CROSS-SECTIONAL CUTS IN THE INTERNODES OF RESCUE

Stage of plant shaded	Internode				
	1	2	3	4	5
None (check)	4.96	4.95	4.90	3.26	2.28
Emergence to 2-leaf	4.98	5.00	4.98	3.14	2.27
2-leaf to 4-leaf	4.92	4.97	4.90	3.48	2.20
4-leaf to boot	4.42**	4.10**	3.52**	3.74†	3.20††
Emergence to boot	3.82**	3.42**	3.88**	3.85††	2.76†

\*Significantly lower than check

†Significantly higher than check

than the check. Shading between the 4-leaf and boot stages also increased the solidness of Internode 5, while shading from the boot to the heading stage completely reduced the solidness of this internode, although the check was so hollow that a significant difference could not be obtained. There appeared to be no consistent relationship between length of an internode and its solidness.

Normally, in Rescue, the pith cavity extends down from the upper node in each internode, but when Rescue was shaded from the boot to the heading stage, a large cavity developed in the lower part of Internode 3. This occurred because approximately 75 per cent of Internode 3 had already developed at the boot stage and the lower 25 per cent developed after the cage was placed over the plants.

Shading from the boot to the heading stage greatly increased the brittleness of the stems. Almost all of the stems that received this treatment were broken near the third internode at maturity whereas most of the stems from the other treatments were still in good condition. This suggests that the period from the boot to the heading stage may be the most critical period in determining the characteristics of the straw that are associated with lodging resulting from excessive reduction in light intensity.

#### *Comparison of the Two Techniques for Measuring Solidness*

The data obtained independently by the two methods were very similar (Tables 2 and 3). A correlation coefficient of 0.89, significant at the 1 per cent level, was obtained between the data from the cuts made in the middle of the internodes and the percentage solidness of the internodes. Similarly, the correlation coefficient of 0.91 between the cuts at the top of the internodes and the percentage solidness was equally significant. Thus, the diameter of the pith cavity is significantly related to the length of the pith cavity and the measurement of either should give a good index of the solidness of an internode. However, cross-sectional cuts can be made much more quickly than the more laborious method of measuring the length of the internode and of the pith cavity. In a few cases significant differences between the treatments and the control were obtained with one method and not the other. Internode 4 showed a significant difference from the control only in the diameter of the pith cavity in plants shaded from the 4-leaf to the boot stage and from emergence to the boot

stage. However, the cavity at the top of Internode 4 was significantly more hollow, while that at the middle was significantly more solid than at the same locations in the unshaded stems. Thus, the amounts of solidness at the two locations appear to nullify each other in respect to the sawfly resistance of the internode, and the failure of the longitudinal measurements to pick out this effect is probably not too serious. In the treatment where the plants were shaded from the 2-leaf to the 4-leaf stage, Internode 1 was significantly more hollow only in the length of the pith cavity (Table 2). It appears that the method of measuring the length of the pith cavity is more sensitive at the upper levels of solidness, as a decrease in the cross-section index of about 25 per cent was accompanied by a drop in the longitudinal index of about 80 per cent. Conversely, the cross-sectional method is more sensitive at the lower levels of solidness. This indicates that the pith cavity starts to lengthen before it increases in diameter but that after the cavity has reached about its maximum length, its diameter continues to enlarge.

Whichever method is used to determine the solidness of the various internodes, the lumping together of all the ratings to obtain a single rating for the whole stem is of doubtful value. Most of the sawfly eggs are generally laid in the lower three internodes. As sawfly larvae can complete their development in one internode, individuals from eggs laid in the lower three internodes of solid stems may tunnel only in these internodes. However, larvae from eggs laid in the upper internodes must tunnel through the lower internodes. Thus, the lower internodes are generally more important in the resistance of a stem to sawflies. Combining the solidness ratings of the upper internodes with that of the lower internodes may give a false picture of the sawfly resistance. This applies in particular to the rating by the cross-sectional method, which includes three cuts in the top internode and two cuts in each of the other internodes.

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# THE INFLUENCE OF THE EXTRACT OF SOME CROPS AND SOIL RESIDUES ON GERMINATION AND GROWTH<sup>1</sup>

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## ABSTRACT

Six plant species were germinated in sand wetted with water extracts of tissues of five field crops and three soils. Deionized water was used as a check. The study was conducted using standard seed germination techniques.

After 7 to 10 days all of the germinated seeds were harvested and measurements made of the shoot and root lengths.

Alfalfa extract caused the greatest reduction in shoot and root length as well as in percentage germination. It caused the greatest increase in the time required for germination. Timothy extract was not quite as harmful as the alfalfa. Extracts of oats, corn and potatoes were still less harmful with potato extract causing the least effect.

The soil extracts generally had very little effect when compared with deionized water.

Plant species showed marked differences in tolerance to the extracts, alfalfa being the most resistant and timothy the least.

Where water alone was used rate of germination and per cent germination were as high or higher than with the other extracts, but the root and shoot lengths were not always the greatest.

## INTRODUCTION

Organic materials in the surface of the soil may have various effects on germinating seeds and growing plants. The materials may be absorbed and cause a stimulating or a depressing effect in the seeds and plants, or may have no effect. It is also possible that they may cause changes which go unnoticed, such as modifications in the nutrient content of the plants. These organic materials may also influence the development of pathogens.

Early work on the injurious effects of preceding crops (14, 16) suggested nutrient deficiency to be important. Secretion of toxic substances by growing plants, production of these substances in decomposition of plant residues, and development of pathogenic organisms were also considered.

More evidence has accumulated recently to show many plants contain germination and growth inhibitors (1, 2, 3, 4, 6, 10, 12, 13, 18) and also to show that toxic compounds are produced in the decomposition of crop residues in the soil (1, 4, 15). The effects of crop residues on the development of soil pathogens have also been investigated (7, 10, 12, 15, 17, 19).

Categorization of the toxic compounds has been made by investigators in some of the work (e.g. 10, 12, 13) but, for the most part, the compounds have remained unidentified.

Our interest in the work reported here arose from the observation that there were marked differences in the rate of germination and early growth of a number of plant species planted in different soils, even when they were maintained at the same temperature and similar moisture tensions. The purpose of the study was to investigate the effects of crop residues on the germination and growth of six plant species. It was decided to leave a critical examination of the soils themselves to a later date.

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## MATERIALS AND METHODS

Extracts of five crop residues and three soils were used in this study. Deionized water was used as the check.

The crop residues extracted were:

Alfalfa hay (about 50 per cent bloom)  
Timothy hay (about 50 per cent bloom)  
Corn stover (mature)  
Oat straw (mature)  
Potato vines (mature).

The soils extracted were:

North Gower clay loam (previously in grass sod)  
Grenville loam (previously cropped to oats)  
Castor silt loam (previously cropped to oats).

Dried and ground plant material and distilled water, at the ratio of 1 to 10, were mixed in a Waring blender for 10 minutes and then filtered through cheesecloth to remove the fibre.

The soil was extracted in the same manner, using a ratio of 1 to 5, soil to water.

Seeds of six plant species were then placed in pure quartz sand (8), wetted to approximately 50 per cent water-holding capacity with the extracts and germinated in custom-made temperature controlled germination cabinets with Barber-Coleman controls. The plant species used and preparation for germination were as follows:

Plant species	Number of seeds per box	Ml. of extract per box*	Cups of sand	
			Under seed	Over seed
Alfalfa				
<i>Medicago sativa</i> L.	100	30	3	2
Timothy				
<i>Phleum pratense</i> L.	100	30	5	0
Oats				
<i>Avena sativa</i> L.	100	30	2½	2½
Soybeans				
<i>Glycine max</i> (L.) Mert.	50	60	2½	2½
Peas				
<i>Pisum sativa</i> L.	50	60	2½	2½
Corn				
<i>Zea mays</i> L.	50	60	2½	2½

\* Each box contained 5 cups of sand; each cup weighed approximately 135 gm.

Corn and soybeans were germinated at 25° C. (77° F.) and peas, oats and alfalfa were germinated at 20° C. (68° F.) all in the dark. Timothy was germinated under conditions of alternating temperature, i.e., 14 hours at 20° C. and 6 hours at 30° C. (86° F.) with 2 hours for the transition in each direction. Lights were automatically turned on for the 8-hour high temperature period. These germination techniques are standard for the Seed Research Laboratory (9).

TABLE 1.—THE EFFECTS OF EXTRACTS OF SOME SOILS AND FIELD-CROP RESIDUES ON SEED GERMINATION AND GROWTH OF ALFALFA

Measurement	Extract									
	Water	Oat	Castor	Grenville	N. Gower	Potato	Corn	Timothy	Alfalfa	
Days to germinate	3.0	3.0	3.0	3.0	3.0	3.0	3.5	3.5	4.0	
Per cent germination	Alfalfa 57.0	Timothy 73.0	Castor 74.5	N. Gower 76.3	Grenville 77.3	Oat 77.8	Potato 78.3	Water 79.8	Corn 80.8	
Root length—mm.	Alfalfa 11.5	N. Gower 19.7	Grenville 21.1	Castor 21.8	Corn 22.1	Potato 22.8	Timothy 23.0	Oat 23.5	Water 24.7	
Shoot length—mm.	Alfalfa 40.3	Corn 50.7	Timothy 53.8	Water 53.9	Castor 57.6	N. Gower 58.6	Grenville 59.5	Potato 60.2	Oat 60.7	
Shoot-root ratio	Water 2.2	Corn 2.3	Timothy 2.3	Potato 2.6	Castor 2.6	Oat 2.6	Grenville 2.8	N. Gower 3.0	Alfalfa 3.5	

Note: Any two means not underscored by the same line are significantly different.

TABLE 2.—THE EFFECTS OF EXTRACTS OF SOME SOILS AND FIELD-CROP RESIDUES ON SEED GERMINATION AND GROWTH OF TIMOTHY

Measurement	Extract									
	Castor	Water	Grenville	N. Gower	Corn	Potato	Oat	Alfalfa	Timothy	
Days to germinate	3.3	3.5	3.5	3.5	4.4	5.0	6.0	7.0	8.0	
Per cent germination	Alfalfa 3.0	Timothy 5.5	Potato 12.3	Oat 27.5	Corn 48.3	Castor 54.5	Water 59.0	Grenville 59.3	N. Gower 69.5	
Root length—mm.	Alfalfa 0.25	Timothy 1.7	Potato 8.4	Oat 9.7	Corn 16.6	Water 30.5	Grenville 31.2	N. Gower 32.5	Castor 32.5	
Shoot length—mm.	Alfalfa 1.8	Timothy 3.2	Potato 10.9	Corn 15.2	Oat 16.5	N. Gower 18.8	Castor 18.8	Water 19.7	Grenville 20.2	
Shoot-root ratio	Water 0.6	Grenville 0.6	N. Gower 0.6	Castor 0.6	Corn 0.9	Potato 1.3	Oat 1.7	Timothy 1.9	Alfalfa 7.2	

Note: Any two means not underscored by the same line are significantly different.



There were four replicates of each extract on each plant species.

Readings were taken every 24 hours to assess the rate of germination. A final reading was taken for per cent germination on the 7th day after planting for all species except timothy where the final reading was made on the 10th day. After the final reading was made, the seedlings were removed and root and shoot lengths measured for each germinated seed.

## RESULTS

The data are presented in Tables 1 to 6. The method of comparison was that of Duncan's (5) in which any two means not underscored by the same line are significantly different, and any two means underscored by the same line are not significantly different.

### *Rate of Germination*

Germination of all species was generally faster with deionized water than it was with any of the extracts. Alfalfa extract caused the greatest delay in germination of all species with only one exception—germination of timothy was delayed the longest where timothy extract was used. The effect of timothy extract was similar to that of alfalfa extract with the other species.

The crop extracts all delayed germination when compared to water except in two cases, namely oat extract on alfalfa seeds and potato extract on oat seeds (Tables 1 and 3). In these two instances there was no difference in effect between the extract and water alone.

In only one instance did the extracts of the soils delay germination. This occurred where peas were grown (Table 5). All three soil extracts delayed germination by a half-day as compared to water alone.

### *Per cent Germination*

Alfalfa extract significantly reduced the per cent germination of alfalfa below that of the other extracts (Table 1). Germination of timothy seeds was reduced by all the crop extracts and most severely by those of alfalfa, timothy and potato (Table 2). There was essentially no effect of the soil extracts when compared to that of water, on germination of timothy.

The effects of extracts on germination of the other species were not statistically significant.

### *Root and Shoot Lengths*

The lengths of the roots and shoots of germinated seedlings were considered to be functions of the effect of the extracts on time of germination plus their effects, either stimulating or inhibiting, on the new tissues themselves. Hence, where there are differences in general order of effects of the treatments on root and shoot lengths as compared to days to germinate, it was probably due to the influence of the extracts on the germinated seedling.



TABLE 3.—THE EFFECTS OF EXTRACTS OF SOME SOILS AND FIELD-CROP RESIDUES ON SEED GERMINATION AND GROWTH OF OATS

Measurement	Extract								
	Water 3.5	Castor 3.5	Grenville 3.5	N. Gower 3.5	Potato 3.5	Oat 4.0	Corn 4.0	Timothy 4.5	Alfalfa 5.5
Days to germinate									
Per cent germination	N. Gower 82.5	Water 84.8	Alfalfa 85.3	Grenville 86.3	Oat 86.5	Timothy 87.0	Potato 87.5	Corn 87.8	Castor 88.5
	Alfalfa 10.6	Timothy 87.7	Corn 89.1	Oat 89.2	Potato 96.9	Castor 106.6	Grenville 107.0	N. Gower 108.1	Water 111.0
Root length—mm.	Alfalfa 27.1	Corn 62.3	Potato 67.8	N. Gower 69.7	Grenville 71.4	Oat 72.4	Timothy 73.6	Castor 74.0	Water 80.7
	N. Gower 0.6	Water 0.7	Castor 0.7	Grenville 0.7	Corn 0.7	Potato 0.7	Timothy 0.8	Oat 0.8	Alfalfa 2.6
Shoot-root ratio									

Note: Any two means not underscored by the same line are significantly different.

TABLE 4.—THE EFFECTS OF EXTRACTS OF SOME SOILS AND FIELD-CROP RESIDUES ON SEED GERMINATION AND GROWTH OF SOYBEANS

Measurement	Extract								
	Water 3.0	Castor 3.0	Grenville 3.0	N. Gower 3.0	Oat 3.5	Potato 4.0	Corn 4.5	Timothy 4.5	Alfalfa 5.5
Days to germinate									
Per cent germination	Potato 89.0	N. Gower 89.0	Timothy 90.5	Alfalfa 91.0	Water 91.5	Castor 92.5	Oat 93.0	Corn 93.0	Grenville 93.0
Root length—mm.	Alfalfa 61.7	Timothy 143.8	Oat 145.0	N. Gower 152.6	Water 152.8	Corn 152.8	Potato 155.8	Castor 162.7	Grenville 164.9
Shoot length—mm.	Alfalfa 43.1	N. Gower 71.0	Timothy 71.2	Oat 72.5	Corn 80.7	Water 89.6	Castor 89.9	Potato 90.7	Grenville 97.3
Shoot-root ratio	N. Gower 0.4	Timothy 0.5	Oat 0.5	Corn 0.5	Water 0.6	Castor 0.6	Potato 0.6	Grenville 0.6	Alfalfa 0.7

Note: Any two means not underscored by the same line are significantly different.

The extract of alfalfa caused the greatest reduction in lengths of roots and shoots of all the species planted. The depressing effects were significantly greater than those of all the other extracts, except where timothy was grown (Table 2), and was generally greater by far than what would be expected from a consideration of the delay in germination.

Next to the alfalfa extract, timothy extract reduced root growth of all species most, except with peas where the inhibiting effect of corn extract was similar to that of the timothy extract (Table 5). Timothy extract affected shoot growth of all the plant species except oats, more seriously than did the extracts of oats, corn or potatoes. Where oats were grown (Table 3) the shoots were significantly longer with timothy extract than with the other crop extracts.

Of the other three crop extracts, potato extract was generally "better" (that is, roots and shoots were longer) than that of oats or corn, and oat extract was usually "better" than that of corn.

Generally speaking, the soil extracts had only a slight effect on the root and shoot lengths when compared with deionized water. Of the three soil extracts, that of the North Gower clay loam caused depressing effects most frequently. This soil sample had been taken from a broken grass sod, whereas the other two were taken from soil previously cropped to oats.

Where water alone was used to wet the sand, the root and shoot lengths were not always the greatest. This was quite evident with alfalfa shoots (Table 1), soybean roots (Table 4), pea roots and shoots (Table 5) and corn shoots (Table 6). In these instances the measurements were significantly less with water than they were for some of the other extracts. It is possible that the "better" extracts in these cases promoted the growth of the roots and/or shoots since it is not suspected the water contained harmful compounds.

Plant species showed marked difference in tolerance to the different extracts. All plant species were harmed the most by the alfalfa extract, but there was some exchange of position of tolerance of the species to the other extracts used. For example soybean root and shoot lengths were adversely affected by the oat extract, but alfalfa plants showed essentially no effect; root and shoot lengths of timothy were depressed by potato extract but alfalfa and peas showed little or no effect.

If the plant species are arranged in general order of decreasing resistance to the toxic effects of the extracts (as indicated by the ratio of the largest root length to the smallest and the ratio of the largest shoot length to the smallest), they are as follows: alfalfa, corn, soybeans, peas, oats, timothy.

#### *Shoot : Root Ratios*

The shoot : root ratios are given to show the effects of the extracts on the shoots compared to the roots of the different species.

The roots were generally more affected by the extracts than the shoots, that is, there was a differential action of extract on root and shoot. Consequently, those extracts having the greatest effect on the shoot and root lengths usually produced the largest shoot : root ratio.

Shoot-root ratio

Note: Any two means not underscored by the same line are significantly different.

TABLE 5.—THE EFFECTS OF EXTRACTS OF SOME SOILS AND FIELD-CROP RESIDUES ON SEED GERMINATION AND GROWTH OF PEAS

Measurement	Extract									
	Water	Castor	N.Gower	Grenville	Oat	Corn	Potato	Timothy	Alfalfa	Timothy
Days to germinate	3.0	3.5	3.5	3.5	4.0	4.5	4.5	4.5	5.0	5.0
Per cent germination	Potato 98.0	Water 98.0	Grenville 98.5	Oat 99.0	Corn 99.0	Castor 99.5	N.Gower 99.0	Alfalfa 100	Timothy 100	Timothy 100
Root length—mm.	Alfalfa 20.4	Water 78.2	N.Gower 80.9	Castor 88.1	Grenville 88.2	Corn 90.8	Timothy 94.4	Oat 113.4	Potato 118.3	Potato 118.3
Shoot length—mm.	Alfalfa 29.4	Timothy 43.1	Corn 57.5	Oat 59.1	Grenville 65.8	Water 76.8	N.Gower 77.1	Castor 86.3	Potato 95.8	Potato 95.8
Shoot-root ratio	Timothy 0.46	Oat 0.52	Corn 0.63	Grenville 0.75	Potato 0.81	N.Gower 0.95	Castor 0.98	Water 0.98	Alfalfa 1.4	Alfalfa 1.4

Note: Any two means not underscored by the same line are significantly different.

TABLE 6.—THE EFFECTS OF EXTRACTS OF SOME SOILS AND FIELD-CROP RESIDUES ON SEED GERMINATION AND GROWTH OF CORN

Measurement	Extract									
	Water	Castor	Grenville	N.Gower	Potato	Corn	Timothy	Oat	Alfalfa	Alfalfa
Days to germinate	3.0	3.0	3.0	3.0	3.0	3.5	3.5	3.5	4.0	4.0
Per cent germination	Castor 98.0	N.Gower 98.5	Corn 99.0	Potato 99.0	Timothy 99.0	Grenville 99.5	Alfalfa 99.5	Oat 99.5	Water 100	Water 100
Root length—mm.	Alfalfa 103.6	Timothy 161.7	N.Gower 163.1	Corn 164.5	Potato 176.7	Oat 182.0	Water 184.7	Grenville 185.6	Castor 186.6	Castor 186.6
Shoot length—mm.	Alfalfa 50.6	Corn 71.3	Timothy 77.1	N.Gower 77.9	Grenville 85.7	Potato 89.7	Water 90.8	Castor 93.2	Oat 105.0	Oat 105.0
Shoot-root ratio	Corn 0.43	Grenville 0.46	Castor 0.47	Timothy 0.48	N.Gower 0.48	Water 0.49	Alfalfa 0.49	Potato 0.51	Oat 0.58	Oat 0.58

Note: Any two means not underscored by the same line are significantly different.

The ratios where alfalfa extract was used were significantly greater than for the other extracts for all the plant species except corn (Table 6) in which case the oat extract caused the largest ratio. The extracts of oats and timothy affected the roots of oats and timothy relatively more than the shoots when compared with the other extracts (excepting alfalfa). On the other hand, the ratios with these two extracts were smaller with peas than those for the other extracts.

The potato extract had an effect similar to that of water for all plant species except timothy in which case it produced a higher shoot : root ratio than water.

The ratios with the soil extracts were generally similar to those with water.

#### *Effect of Each Species on Itself*

The data concerning the effects of the extracts of different crops on themselves are assembled in Table 7 and are given as the ratio of the measurement with the extract divided by the measurement with deionized water.

The higher the value for per cent germination, root length, and shoot length, the less was the effect of the extract compared with water. Timothy was most severe on itself while corn and oats had much less effect on themselves. Extract of alfalfa caused the greatest proportional decrease in its root length but the effect on its shoot length and per cent germination was less.

The ratio for the number of days to germinate show timothy to be very severe on itself. The extracts of the other crops had less effect on themselves.

#### DISCUSSION

The results reported here indicate that all of the crop residues studied contained substances toxic to at least one of the plant species grown. These data are similar to those of other workers (7, 10, 11, 12, 13) in suggesting that the inhibition is due to compounds present in the plant extract, but are different to those of Patrick and Koch (15). These investigators found that timothy, rye or corn do not contain substances having inhibiting effects on the respiration of tobacco seedlings. They suggest that most of the toxic effects reported in their paper were due to substances formed during decomposition of the plant residues in the soil. They also found that the toxic substances which they extracted from soils containing

TABLE 7.—THE EFFECTS OF THE EXTRACT OF SOME CROPS ON THEMSELVES

Measurement	Ratio of: $\frac{\text{measurement with the extract}}{\text{measurement with water}}$			
	Alfalfa	Timothy	Oats	Corn
Days to germinate	1.33	2.29	1.14	1.17
Per cent germination	0.71	0.09	1.02	1.00
Root length	0.47	0.56	0.80	0.89
Shoot length	0.75	0.16	0.90	0.78

decomposing materials were relatively non-specific in their action in that they affected tobacco, timothy and barley in about the same way. Our plant extracts were quite specific in their effect since the plant species were often affected differently by the extracts. It is possible that the incubation process in the soil as done in the work of Patrick and Koch (15) could account for the difference in results.

Techniques in a study of this nature may be very important. Differences in results may be due to plant species used, stage of maturity at which plants are taken, proportion of plant material to water in the extractions, the thoroughness of the extraction and the concentration of the extract around the germinating seeds. Microbial contamination is also a likely factor. Unlike some of the studies of this nature, the materials used here were not sterilized. It was felt this process might alter the toxicity of the extracts. The use of autoclaved soil has, in itself, affected the growth of plants. The possibility cannot be discounted, therefore, that some of the inhibiting effects obtained were due to differences in populations of pathogens in the systems and/or to toxic substances produced in decomposition of the organic materials in the extracts during the time the seeds were germinating.

Under field conditions some crop residues decompose more readily than others so that toxic compounds contained in the residues, or produced in their decomposition, may not remain long in the soil. In any case even compounds resistant to decomposition ultimately disappear from the soil. This is of particular interest in view of the results obtained with the alfalfa extract. That alfalfa is a valuable crop in a rotation has long been recognized. The toxic compounds in the tissues and those formed in decomposition must disappear quite readily from the soil. Coupled with this, and perhaps of predominant importance, is the nutritional effect of alfalfa when decomposed.

#### ACKNOWLEDGEMENT

Appreciation is expressed to D. N. Bryant, who performed the statistical analyses.

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# EFFECTS OF SEVIN ON PHYTOPHAGOUS MITES AND PREDATORS IN AN ONTARIO PEACH ORCHARD

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[Received for publication June 25, 1959]

## ABSTRACT

Numbers of the European red mite (*Panonychus ulmi* Koch) increased but those of the brown mite (*Bryobia arborea* Morgan & Anderson) and the peach silver mite (*Vasates cornutus* Banks) decreased after sprays of Sevin (*N*-methyl-1-naphthyl carbamate) were applied to peach trees. Sevin practically eliminated the predacious mite *Typhlodromus rhenanus* Oudem., and larvae and pupae of *Stethorus punctillum* Weise, and greatly reduced the numbers of adults of *S. punctillum*, chrysopid larvae, and spiders.

## INTRODUCTION

The recently introduced insecticide Sevin (*N*-methyl-1-naphthyl carbamate)<sup>2</sup> is toxic to a wide range of insects, including the oriental fruit moth, *Grapholitha molesta* (Busck), but is relatively innocuous to some species of tetranychid mites, according to the manufacturer's literature and informal reports and communications from entomologists in the United States and Canada and to some preliminary experiments by the staff of the Vineland Station laboratory in 1957. These properties suggested that its use in peach orchards might have the effect, similar to the well-known one of DDT, of promoting increase in numbers of the European red mite, *Panonychus ulmi* (Koch), and other phytophagous mites. This is a report of an experiment in 1958 on the effects of Sevin on various phytophagous and predacious mites and predacious insects, as part of a long-term study of the indirect effects of pesticides on the peach orchard fauna.

## PROCEDURE

The experiment was carried out in two 40-tree plots in the Boothman orchard of mature Veteran peach trees at the Ontario Horticultural Experiment Station, Vineland Station, Ontario. Plot A, which had not received any pesticides for the preceding 4 years, except an annual "dormant" application of bordeaux mixture, was used to test the effects of Sevin on phytophagous mites, predacious phytoseiid mites, and other predators. Plot B, which had been sprayed with DDT in 1956 and 1957 and also received one application of 2 pounds of 50 per cent DDT wettable powder per 100 gallons on June 9, 1958, was used chiefly to determine the effects on the coccinellid *Stethorus punctillum* Weise, a predator of mites, which became common as the European red mite became abundant.

In Plot A, 8 trees taken at random through the plot were sprayed with 2 pounds of 50 per cent Sevin wettable powder per 100 gallons of water on June 9, July 3 and 22, and August 13 by means of a hydraulic sprayer and a hand-operated gun. Eight other trees, taken at random in

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the same block, were sprayed with water alone on the same dates as checks. The check trees were protected from Sevin spray drift by large cotton sheets held on poles.

In Plot B, 5 trees taken at random were sprayed once on August 13 with Sevin at the same rate and in the same manner as in plot A, and 5 other trees were sprayed with water alone.

Samples of 10 twigs from each tree in Plot A were taken on May 29. Each twig bore a terminal and two lateral shoots of the current season's growth, which was at that time approximately  $1/4$  to  $3/4$  inches long. Both the bark and leaves of the twigs were carefully examined under a binocular microscope for phytoseiids and other mites. The phytoseiids tend to occur mainly on the bark. Similar samples of twigs were collected on June 29, but only the bark and the 2 basal leaves of each shoot (i.e., 60 leaves per tree) were examined. Phytophagous mites were also counted on 50 leaves taken at random from each tree at approximately 2-week intervals from July 10 to August 22 and counted with a Henderson-McBurnie mite-brushing machine. Larger predators such as chrysopid larvae and spiders were sampled on July 9, 21, and 29, August 11 and 22, and September 3 by jarring three branches of each tree over a cotton sheet.

In Plot B, the immature stages of *Stethorus punctillum* were sampled by collecting and brushing 50 leaves at random per tree on August 18, and the larvae and adults of this species and chrysopid larvae by jarring on August 22 and September 3.

Numbers of the European red mite and the brown mite, *Bryobia arbores* Morgan & Anderson, were transformed to logarithms and statistically analysed by Student's *t* test. Numbers of other species were analysed by the rank method according to Snedecor (5, p. 117, sect. 5.10). The levels of significance (0.1, 1, or 2 per cent) of the differences between the numbers from treated and untreated trees are given in parentheses after appropriate pairs of numbers in the following account.

### RESULTS

Increase of the European red mite on all trees in Plot A was slow at first, as in other orchards during the cool first half of the summer, but by August 22 the numbers on the Sevin-sprayed trees were more than three times as great as those on the water-sprayed ones (Table 1).

TABLE 1.—AVERAGE NUMBERS OF THE EUROPEAN RED MITE PER 50 LEAVES ON PEACH TREES SPRAYED WITH SEVIN AND WITH WATER ON JUNE 9, JULY 3 AND 22, AND AUGUST 13

Date	Sevin		Water	
	Eggs	Motile stages	Eggs	Motile stages
June 29	—	0.2	—	0.5
July 10	5.0	5.5	4.5	3.2
July 23	61.8	1.4	39.2	1.8
Aug. 7	219.9 <sup>1</sup>	52.0 <sup>2</sup>	72.1	19.4
Aug. 22	559.2 <sup>1</sup>	332.0 <sup>1</sup>	201.1	105.6

<sup>1</sup>Significantly greater than for water-sprayed trees at the 1% level

<sup>2</sup>Significantly greater than for water-sprayed trees at the 2% level

Average numbers of motile stages of the brown mite on the twig samples (May 29 and June 27) and on samples of 50 leaves (subsequent dates) from Plot A were:—

Treatment	May 29	June 27	July 10	July 23	Aug. 7	Aug. 22
Sevin	40	18	10	22	2	0
Water	37	14	26	95	93	133

The numbers on the Sevin-treated trees were significantly less than on the water-sprayed checks at the 5 per cent level on July 10 and at 1 per cent on subsequent dates.

As usual in local orchards, few or no peach silver mite, *Vasates cornutus* (Banks), occurred in the leaf samples in Plot A until August. On August 22 the Sevin-treated trees averaged 195 and the checks 4,356 per 50 leaves (0.1 per cent).

Total numbers of all stages of phytoseiids in the twig samples from Plot A on May 29 and June 27 respectively were 67 and 6 for the Sevin-treated trees and 64 and 330 (1 per cent) for the checks. Over 85 per cent were *Typhlodromus rhenanus* (Oudms.). Numbers of these mites in the leaf samples were very small and irregular, but the mites were consistently absent in those from the Sevin-treated trees.

In Plot B, which alone contained appreciable numbers of *Stethorus punctillum*, the leaf samples from the Sevin-treated trees yielded totals of 1 living, newly hatched larva, 4 dead larvae, and 12 dead pupae; those from the checks, 21, 1, and 1 respectively, as well as many pupal exuviae from which adults had emerged. A total of 75 living larvae were jarred from the check trees but none from the Sevin ones. Sixty-four adults were jarred from the check trees and twenty-seven from the Sevin-treated ones. The difference is significant at the 5 per cent level.

Totals of chrysopid larvae jarred from the Sevin-treated and check trees in Plot A were respectively 9 and 44 (1 per cent) and in Plot B, 1 and 15 (5 per cent). On the Sevin-treated trees in Plot A all larvae were *Chrysopa plorabunda* Fitch, and on the checks, 34 were *C. plorabunda*, 9 *C. rufilabris* Burm., and 1 *C. oculata* Say.

In Plot A, a total of 115 spiders were jarred from the Sevin trees and 608 from the checks (1 per cent). Over 75 per cent were the young of *Theridion murarium* Emerton and most of the remainder were those of *Philodromus cespiticolis* Walckenaer.

#### DISCUSSION

The increased populations of the European red mite that followed the application of Sevin in this experiment may be compared with similar increases following the use of DDT in the same and other peach orchards. (4). Although the average density on the Sevin-treated trees reached a level considerably greater than that on the checks, it was actually not very great, very probably because of the exceedingly low initial density. DDT likewise did not cause a very great immediate increase in this orchard in plots where the initial density of the mite was very low; but its annual use

eventually caused a much greater increase, and in some other orchards DDT caused a much quicker and greater increase. It is, therefore, likely that Sevin would also raise the mite density to injurious levels after repeated annual use in this orchard, or sooner in orchards where the initial density was greater.

Both Sevin and DDT virtually exterminated the phytoseiids. Lord (1) and earlier unpublished work by the present authors confirm this result with DDT. *Stethorus punctillum*, which is susceptible to DDT in all stages except the egg (2), was also killed by Sevin. Although some adults of this species were collected on the Sevin-sprayed trees, they may have moved to the sprayed trees from surrounding unsprayed ones. Sevin greatly reduced the numbers of chrysopid larvae, including those of *C. plorabunda*: the latter species is very tolerant of DDT (3). It is difficult to compare the effect of Sevin with that of DDT on spiders because experiments conducted at Vineland Station with the latter insecticide have been inconclusive owing to the usual scarcity of spiders in peach orchards; the abundance of *T. murarium* in the plot used for the Sevin experiment was unique in the authors' experience. However, Sevin is at least as toxic to spiders, if not more so, than DDT. All the changes in population densities of the various predators caused by Sevin in this experiment would undoubtedly be intensified in large sprayed blocks where movement from untreated to treated trees could not occur.

Although all of the predators mentioned above have been observed by the authors to prey upon the European red mite, the exact role played by any one, or indeed the entire complex, in the control of the mite has not been elucidated. Nevertheless, the great reduction in the numbers of predators caused by Sevin must have been the major, if not the sole, factor in the increase of the mite.

In its suppression of the brown and the peach silver mite, Sevin differs markedly from DDT, which promoted increased numbers of the former species and did not reduce numbers of the latter in investigations by the present authors (4). These effects of Sevin, however, have little practical importance in the Niagara district because neither species is a serious pest in peach orchards there.

#### ACKNOWLEDGEMENT

The authors wish to acknowledge the co-operation of W. H. Upshall, Director, Ontario Horticultural Experiment Station, Vineland Station, who allowed the use of the orchard.

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## NOTE ON THE OCCURRENCE OF BINUCLEATE PYCNIOspores IN SPECIES OF *PUCCINIA*<sup>1</sup>

Anomalous spore forms in species of *Puccinia* have been observed from time to time, spore colour changes attributed to gene mutations having been noted most frequently (2, 4, 5). Abnormal nuclear numbers have been reported in urediospores (3), and the evidence to be presented here indicates that such abnormalities also occur in low frequencies in pycniospores.

A large number of pycniospores of *P. graminis* Pers. and *P. helianthi* Schw. were examined cytologically to determine their nuclear condition. Staining was accomplished with either aceto-carmin or aceto-orcin. Normal pycniospores are haploid and uni-nucleate. In size they average approximately  $1.6 \times 3.8$  microns in *P. graminis* and  $2.2 \times 4.2$  microns in *P. helianthi*. In both species a very low frequency of abnormally large spores (about 1 in 100,000) was observed in every pycnial smear studied. These spores proved to be binucleate (Figure 1). In *P. graminis* the binucleate spores measured approximately  $2.4 \times 5.6$  microns while in *P. helianthi* they were about  $2.4 \times 7.2$  microns. Buller (1) observed a few pycniospores of *P. graminis* that were two or three times the normal length, but the nuclear condition was not determined. It now seems quite likely that such spores were actually binucleate. The present study indicates that a low frequency of binucleate pycniospores can be expected to occur in at least the two species under discussion and it seems probable that they are present in other species as well.

Since pycniospores are normally haploid, the binucleate condition has probably resulted from an additional mitotic division taking place at the time of pycniospore formation. In any case, both nuclei must be genetically identical except for mutations occurring during or after mitosis. Whether these spores are of evolutionary significance has not been determined.

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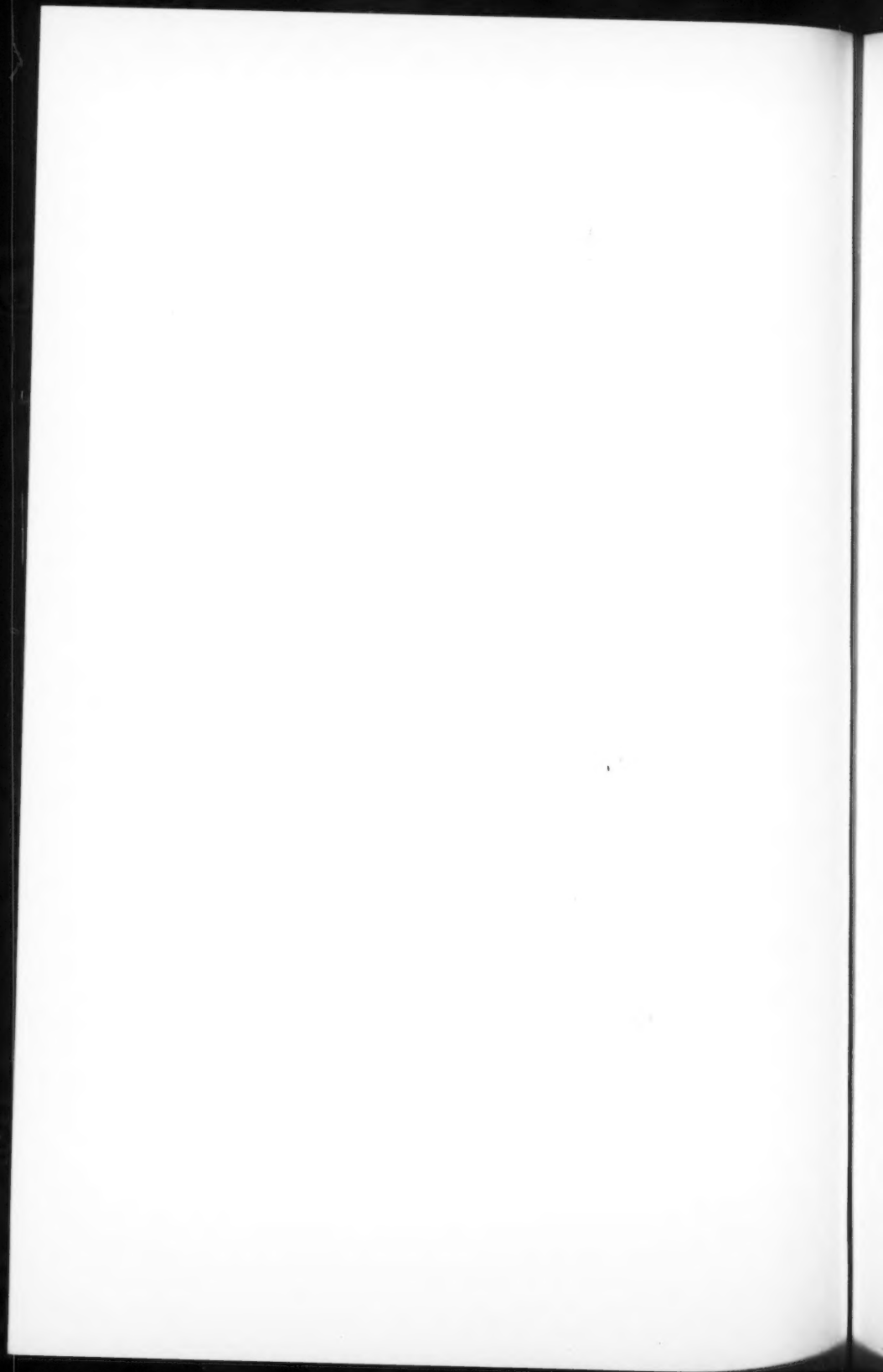
—R. C. MCGINNIS,  
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September 21, 1959

<sup>1</sup> Contribution No. 32 of the Canada Agriculture Research Station, Winnipeg, Man.



FIGURE 1. A binucleate and uninucleate pycniospore of *Puccinia graminis*. 10,000 X.



## NOTE ON "FRONTIER" REED CANARY GRASS

(*Phalaris arundinacea* L.)<sup>1</sup>

This variety, licensed on March 12, 1959, was developed by the Forage Crops Division, Central Experimental Farm<sup>2</sup>, Ottawa, Ont. It is leafy, growthy, and of medium maturity. It is expected that these characteristics will make it more suitable for hay, pasture and silage production than the early, somewhat stemmy sorts now available. Under irrigation, where high soil fertility can be maintained, it gives promise of surpassing species such as timothy, brome and orchard grass in pasture production.

### SOURCE MATERIAL AND BREEDING METHODS

The source material was a collection of native material. The variety was developed through three cycles of breeding, using the maternal line method. In this procedure a restricted polycross type of controlled pollination was used.

The group of initial selections comprised approximately 500 clones. Polycross seed from these plants was used to propagate the nursery for the first cycle. By processing and screening through three complete cycles of this type, highly rated progenies, representative of 47 of the original maternal clones, were secured. These progenies were combined as the synthetic which is now "Frontier".

### VARIETAL CHARACTERISTICS

The variety does not deviate in botanical characteristics from other varieties of the species.

### AGRONOMIC CHARACTERISTICS

*Plant Type*—A tall, leafy variety from 7 to 10 days later in maturity than the common sorts.

*Winter Hardiness*—"Frontier" possesses the general hardiness and adaptability which is characteristic of the species.

*Disease Resistance*—As with the species as a whole, the variety has good resistance to the common leaf and stem diseases.

*Forage Yield*—Under Eastern Canadian conditions this strain has a total yield capacity equal or superior to other known strains.

*Seed Yield*—A heavy seed yielder, equal to other varieties of the species. It does not possess greater non-shattering tendencies.

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<sup>1</sup>Contribution No. 6 from the Genetics and Plant Breeding Research Institute, Canada Department of Agriculture, Ottawa, Ont.

<sup>2</sup>Now part of Genetics and Plant Breeding Research Institute.



## NOTE ON CLIMATIC TRENDS IN THE LOWER PEACE RIVER REGION OF NORTHERN ALBERTA

There are two major climatic factors determining the type of agriculture for any region: precipitation, and temperature together with associated phenomena. The long-term averages and trends of these factors are important in determining the future agricultural activities of a specific area.

Currie (2) stated that, to establish a trend, the observational data must be smoothed to eliminate the obscuring effect of irregular variations. This can be done by computing continuous 10-year sums and plotting them or their averages against the last year taken in the summation. Currie further stated that, to be significant, a trend must have occurred at regular intervals or persisted for a considerable length of time. However, the establishment of new average values for any given climatic factor is of more importance than the actual trend. Trends do not continue indefinitely and may reverse. Significant change in the established average values caused by a reversal in a trend will take several years.

Rainfall at Fort Vermilion, situated in the Lower Peace River region of Northern Alberta, was measured twice daily with a standard 3.5-inch Department of Transport rain gauge. Fresh snowfall was converted to rain equivalent using the relationship of 10 inches of snow equals 1 inch of rain. The growing season was taken arbitrarily as that period from May 1 to August 31 inclusive. Maximum and minimum temperatures were averaged to obtain the mean monthly temperature. The mean annual temperature was determined from the average of the 12 monthly means. The killing frost-free period is the number of days between the last killing frost ( $28^{\circ}\text{F}$ . or less) in the spring and the first killing frost in the autumn. The dividing line between spring and autumn season is July 15.

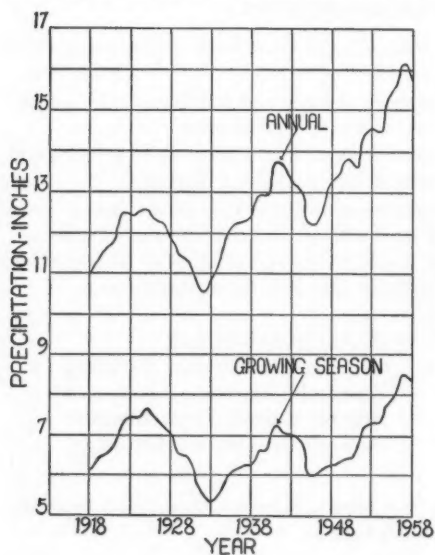


FIGURE 1. 10-year running averages of annual and growing season precipitation at Fort Vermilion, Alberta. These values were obtained by using 10-year accumulated sums for the period 1909-1958.

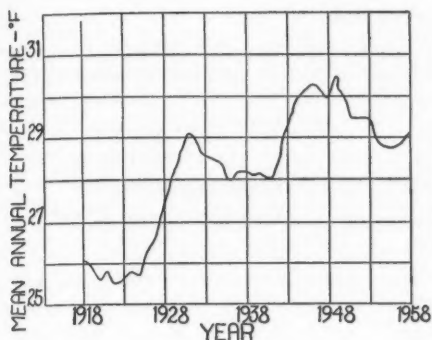


FIGURE 2. 10-year running averages of mean annual temperature at Fort Vermilion, Alberta. These values were obtained by using 10-year accumulated sums for the period 1909-1958.

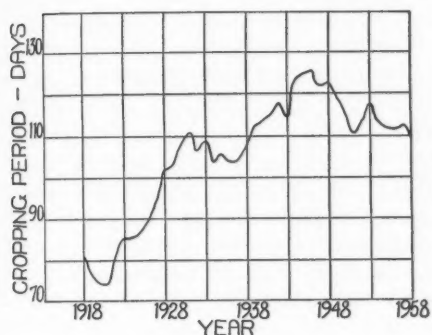


FIGURE 3. 10-year running averages of killing frost-free period at Fort Vermilion, Alberta. These values were obtained by using 10-year accumulated sums for the period 1909-1958.

Decided trends have developed in annual precipitation, mean annual temperature and killing frost-free period at Fort Vermilion despite large year-to-year fluctuations (1).

Both annual and growing season precipitation records at Fort Vermilion show recessions during 1928-1938 and 1943-1948 (Figure 1). Despite these recessions, there has been a decided increase in total annual precipitation at Fort Vermilion during the last 50 years and a less marked increase in the growing season rainfall.

There is a similarity in the trends of mean annual temperature and killing frost-free period at Fort Vermilion (Figures 2 and 3). Mean annual temperature has risen 3° F. over the last 50 years. There appears to be a levelling-off of the upward trend of this factor. The killing frost-free period has increased from the 10-year average of 81 days in the period 1909-1918 to an average of 101 days in the period 1949-1958. This large increase has a marked bearing on varietal and crop usage. A levelling-off of this trend is indicated during the last decade.

It is impossible to determine accurately the future course of these meteorological parameters. However, the foregoing data are useful in assessing new crops and varieties which if grown several decades ago would have been impractical in the Lower Peace River region.

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April 17, 1959

## NOTE ON MERIT SOYBEAN<sup>1</sup>

This newly licensed variety was produced at the Forage Crops Division, Central Experimental Farm, Ottawa, Ontario. Its main attributes include early maturity, high yield, high oil content, resistance to *Phytophthora* root-rot and seed coat mottling.

### SOURCE OF MATERIAL AND BREEDING METHODS

Merit is the progeny of a cross between the varieties Blackhawk and Capital. Blackhawk originated at the Iowa Agricultural Experiment Station, while Capital was produced by the Forage Crops Division. The cross was made at the Soybean Regional Laboratory, Urbana, Illinois, and bulk F<sub>4</sub> seed was sent to the Forage Crops Division in 1950 by J. H. Torrie, (U.S. Dept. Agr.), University of Wisconsin, Madison, Wisconsin. This material was planted in a bulk nursery at Ottawa in 1950 and 1951, following which selections, made chiefly on the basis of maturity, plant type and standability, were carried as single plant progenies until 1955. These were compared with a check variety, Mandarin, for maturity, standability, plant type, disease resistance, resistance to shattering, and yield of seed. Each year the seed was analysed chemically for protein and oil content and iodine number. In 1956 progeny row 2065 was selected for preliminary trial in replicated tests. It was included in comparative tests at Ottawa the two following years. Also in 1957 and 1958 it was entered in the U.S. Regional Zone O tests, which comprised performance trials at approximately 12 locations each year in the United States and Canada. In 1958 it was included in the Quebec Seed Board trials at three locations.<sup>2</sup> On the basis of its fine performance in all these trials, progeny 2065 was granted license as a new variety under the name Merit, March 31, 1959.

### AGRONOMIC CHARACTERISTICS

*Plant Type*—Erect, bushy habit of growth with stems and leaves of medium size. Good stalk strength. Average height, 30 to 36 inches.

*Pubescence*—Grey

*Flower Colour*—White

*Maturity*—117-118 days (at Ottawa), approximately 2-3 days earlier than Comet and 5-6 days earlier than Capital.

<sup>1</sup> Contribution No. 7 from the Genetics and Plant Breeding Research Institute, Research Branch, Canada Department of Agriculture, Ottawa, Ont.

<sup>2</sup> Yield data are recorded in the 1956-58 annual reports of the Forage Crops Division, Experimental Farms Service, Canada Department of Agriculture; results of Cooperative Uniform Soybean Tests 1957-58 U.S.D.A. Regional Soybean Laboratory, Urbana, Ill., and the 1958 Report of the Subcommittee on Corn, Soybeans and Sugar Beets, Quebec Seed Board, Quebec Department of Agriculture.

*Seed Colour*—Seed coat, yellow  
—Hilum, light brown  
—Cotyledons, yellow

*Seed Size*—Small to medium

*Seed Analysis*—Protein, 40 per cent (average, all tests)  
—Oil, 20 per cent (average, all tests)  
Merit is the highest in percentage oil content of varieties within its maturity range.

*Seed Yield*—30-35 bushels per acre (average, all tests)<sup>2</sup>  
Merit has averaged 2-3 bushels per acre higher than Comet and equal to Capital.

*Seed Coat Mottling*—Merit has shown high resistance to mottling.

*Disease Resistance*—Merit has shown resistance to Phytophthora root-rot (artificially inoculated).

#### ADAPTATION

Merit should be well adapted to Zones 3 and 4 in Ontario, also to areas in southwestern Quebec.

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July 14, 1959

## NOTE ON A PLOT IRRIGATOR

Applying water uniformly in precise quantities to vegetables in small-plot irrigation studies is usually difficult with standard sprinkler equipment. The part circle sprinkler, generally used, permits application in only approximate quantities. Moreover, the water stream is purposely broken by sprinkler projections to cause a misty spray which, when delivered from a height of 30 to 36 inches—the standard heights of most riser pipes accommodating sprinkler heads—exposes the irrigation delivery stream to wind play resulting in uneven distribution of water. This can be a markedly adverse factor in plot research.

The problem has been overcome in irrigation studies at Morden with a special plot irrigator illustrated in Figure 1. This device has been highly effective in applying water in precise quantities and in placing it uniformly over the plot area.

The basic parts of the irrigator unit consist of a hand valve, commercial water meter, header pipe and four perforated distributor pipes. The hand valve regulates the quantity of water applied and is located between the supply pipe and water meter. The latter, a "Trident" meter, connected to the centre of the header pipe, provides the measure for precise application of water in gallon quantities.

The water is directed from the meter to the header pipe, a 2-inch diameter galvanized iron pipe provided with "tee" pipe outlets spaced 36 inches apart. Conventional 2-inch aluminum couplers are joined to these outlets with galvanized iron nipples. Gasket cement is used to seal the unions.

Four 15-foot perforated, aluminum pipes, inserted in the couplers and stoppered at the ends with end plugs, distribute the water in the plot to be irrigated. The perforations, 32 in each pipe, are in pairs located at

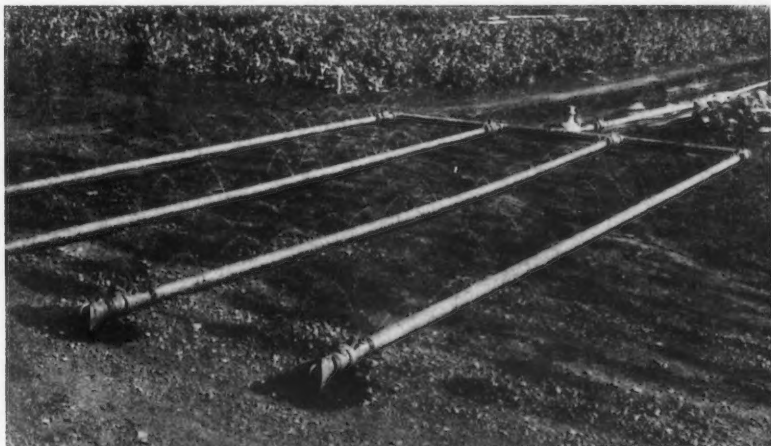


FIGURE 1. Top view of irrigator devised at Morden showing uniform water distribution through pipe perforations.

12-inch intervals beginning 12 inches from the tee. In each pair the perforations are 1 inch apart and astride the centre line of the pipe. The four pipes distribute 1 inch of water in 10 minutes on a 15 x 15 foot square plot, provided the water flow is adequate to permit a 2-inch overlap of the spray arcs from the perforations. This quantity of water represents the maximum amount placed during each irrigation. If more is required, it can be applied immediately or at short intervals, depending on the rapidity with which moisture is absorbed by the soil. The water spray arcs originating from the pipe perforations vary from 10 to 12 inches high and are in unbroken streams. Because of their low height, close proximity to ground level and the solid streams of water, the spray arcs are not markedly affected by wind action.

Two-inch aluminum irrigation tubing is in standard lengths of 20 feet. Accordingly, plots up to 20 x 20 feet square may be irrigated when standard length pipes are used.

The irrigator is in normal operating position at ground level. Most vegetables planted in rows 36 inches apart can be irrigated in this position. However, canning peas, normally planted in 7-inch rows, must be watered with the pipes in a raised position, especially when the pea plants are full grown. Boxes are useful for supporting the pipes or raising them to the required height. Even in this raised position the 12-inch spray arcs from the pipe perforations have not been markedly affected by wind to adversely influence water distribution on the Morden plots.

Complete satisfaction was obtained with the irrigator at Morden in 1959 in irrigation studies with canning peas, beans and sweet corn. Sixty-four plots were watered approximately 5 to 8 times each during the season. The research workers using the device are satisfied with its performance.

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October 1, 1959



#### **Erratum:**

In the article by W. E. Cordukes, D. A. Shearer and D. J. Cooper, published in April, 1959, Vol. 39, No. 2, "*Canadian Journal of Plant Science*", page 129, Table 1, in column headed "Losses—% of total dry matter ensiled", sub-heading "Fermentation", the percentage figure for the ensiling density of 42 lb. per cu. ft. should read **7.8** and not 17.8.

